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THE DIGESTIVE MECHANISM OF ONE OF THE WEST INDIAN 'EYE GNATS', *HIPPELATES PALLIPES* LOEW*

BY

HENRY W. KUMM, M.D., DR.P.H.

(*From the Laboratory of the Jamaica Yaws Commission*)

(*Received for publication* 14 *December*, 1934)

In a study of how long the spirochaetes of framboesia tropica could remain alive in the digestive tract of *Hippelates pallipes*, Kumm, Turner and Peat (1935) found that *Treponema pertenue* retained their motility for a much longer period in the oesophageal diverticulum than in the stomach of the fly. *Hippelates* flies were allowed to feed on infectious yaws lesions and were then dissected at stated intervals after their infecting feed, a separate dark-field examination being made of the contents of the mid gut and oesophageal diverticulum on each occasion. The majority of the ingested *Treponema pertenue* remained actively motile for about seven hours in the oesophageal diverticulum. In the stomach, on the other hand, their motility was lost very rapidly.

It is obvious that, in an investigation of what happens to *Treponema pertenue* after ingestion by these flies, it is necessary to have a clear understanding of the actual anatomy of the digestive system of the insects. And, in a consideration of how yaws might be transmitted by infected *Hippelates* from person to person, a knowledge of the physiology of the alimentary mechanism, particularly of the regurgitation process which produces the 'vomit drop,' is of the utmost importance. Some considerable time and effort was therefore spent in making a careful study of the anatomy and physiology of the digestive system of *Hippelates pallipes*, and the findings will be given in this report.

The name 'eye gnat' or 'eye fly' is in common use in books on medical entomology for flies of the genera *Hippelates*, *Oscinis* and *Siphunculina*. In various places in the United States, *Hippelates pusio* Loew is well deserving of that appelation because of its persistent habits of buzzing and feeding round the eyes. Herms (1926) gave an account of the conjunctivitis produced in the Coachella Valley in Southern California through the agency of this little fly. In Jamaica, on the other hand, the commonest species—*Hippelates pallipes* Loew—has quite different tastes and habits from *H. pusio*, for it prefers to feed on ulcers on the feet and lower extremities rather than round the eyes of human beings. Therefore the name 'eye gnat' does not apply so well to *H. pallipes* as it does to some of the other species of that genus.

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The anatomy of the mouth-parts of *Hippelates pusio*, *Hippelates flavipes*, *Oscinis pallipes* and *Siphunculina funicola* have been carefully described by Graham-Smith (1913). The fly which he called *Hippelates flavipes* is what we are now designating *Hippelates pallipes*, after Aldrich (1931). Because of the previous work by Graham-Smith, no attempt was made in the course of this investigation to study the proboscis of *H. pallipes*. Probably that author's most important conclusion from his work was that the spines of the six pseudo-tracheae in the labella of the proboscis apparently act as cutting instruments capable of producing minute multiple incisions, likely to assist pathogenic organisms carried by the insects in gaining a foothold.

FEEDING HABITS OF *HIPPELATES PALLIPES*

It has already been pointed out that *Hippelates pallipes* feed by preference on the feet and lower extremities. However, under certain conditions and when they are present in very great numbers, they will feed on other parts of the body also. They are attracted principally by ulcers, and feed with great avidity on the exuding sero-purulent material. Some of the other species of the genus *Hippelates* in Jamaica are attracted in large numbers by the mixture of decaying liver and urea in water advocated by Parman (1932) for use as a bait in traps. *Hippelates pallipes* are not attracted by this mixture. This would seem to indicate that, as potential vectors of yaws, *H. pallipes* are probably of more importance than the other species, because of their positive tropism towards ulcers on living men or animals rather than towards decaying dead animal matter.

Hippelates pallipes are attracted in large numbers by certain animals as well as by man. Donkeys, dogs, horses, mules, cows, goats and pigs are all good, particularly if they have chronic ulcers on their legs or bodies. Chickens seem to be useless as bait for these insects. More flies can be caught on a dog wet with water than on an animal with dry fur. In the absence of ulcers on the dog, the *Hippelates* flies will ingest this water, presumably because it is flavoured with the oily secretion of the dog's skin and hair. Often they will feed round the penis of a male dog. We have found what were apparently dog spermatozoa on one occasion in a fly which had been caught, using a male dog as bait.

Adult female *Hippelates pallipes* do not feed on manure, decaying fruit or rotting vegetable matter so far as we have been able to determine. They feed as well if not better in direct sunlight than in the shade. This characteristic is linked up with their marked positive heliotropism. They do not feed when there is a high wind or during rain. But, although feeding in the open may stop while rain is falling, the flies begin again directly any small shower of rain is over. Male flies feed but rarely on ulcer material. It is, therefore, only the females that are of importance as vectors of disease.

Hippelates pallipes when feeding on yaws lesions will crawl underneath the dry scab if necessary to get to fresh sero-purulent material beneath. Sometimes they are caught beneath scabs. If fed on yaws lesions or on ulcers infected

with *Spirochaeta refringens*, they will ingest spirochaetes in great numbers. The actual number of spirochaetes taken in by a given fly appears to depend simply upon the number of organisms present in the exact place in the lesion where the fly obtained its meal. *Hippelates* flies often feed intermittently on different persons and on different lesions.

After a good meal, or during showers of rain or the hottest part of the day, the flies do not usually enter houses or sheds to rest. Instead, they will settle on the under surface of the leaves on trees and shrubs. The leaves of a tree called *Gliricidia maculata* seem to be particularly well liked by them as a resting-place. In trees of this species, *Hippelates pallipes* have been found resting under leaves as high as 18 and 20 feet above the ground.

The amount of blood, pus or serum taken in by them from ulcers is sometimes amazing. With the exception of the tip of the ovipositors and terminal body segments, the abdomen of a fed female *Hippelates* will at times assume the outline of an almost perfect sphere. Microphotographs have been taken of fed and unfed *H. pallipes* (Plate III) to show the magnitude of this distention. Incidentally these photographs give a fairly good idea of the general appearance of these ' eye gnats '—the large head, glistening black thorax and pale legs. The wings also have a characteristic appearance and venation.

ANATOMY OF THE ALIMENTARY TRACT

The technique used for dissecting *Hippelates* flies was described in the paper by Kumm, Turner and Peat (1935), and will not be repeated here. However, one or two slight modifications have been used in attempting to remove the entire digestive system intact. The head was cut off with a dissecting needle with a lancet point, which at the same time severed the oesophagus and the common salivary duct in the neck. Once the oesophagus and common salivary duct had been cut through, it was no longer necessary to break them before removing the remainder of the digestive tract from the abdominal cavity. When the head had been cut off, and a good size slit made in the ventral wall of the abdomen, the organs could then be removed in one of two ways. One was to insert the needle fairly deeply near the anterior end of the abdominal cavity, so that it became caught in the oesophageal diverticulum and that organ was pulled out first. Following it came the duct of the diverticulum attached to the proventriculus, mid gut and the rest, all of which appeared in turn as the oesophageal diverticulum was slowly pulled out and away. Using great care and slow traction it was sometimes possible to remove all the organs in this sequence without rupturing the diverticulum or anything else.

The other process began from the posterior end of the abdomen. The slit in the abdominal wall being carried up to the terminal segments, the needle was then firmly inserted into the chitinous material of the ultimate or penultimate abdominal segment, and traction made on it. This would then pull out first

the rectum and internal genitalia, then the hind gut, malpighian tubules, mid gut and proventriculus, with finally the duct of the diverticulum and the oesophageal diverticulum itself. This latter method had the advantage that the point of the needle, where the maximum traction was exerted, was inserted into a tough chitinous material which was not easily torn. But with this technique it was often impossible to avoid rupturing the intestinal tract at or near the junction of the mid and hind guts, particularly when the former was full of blood.

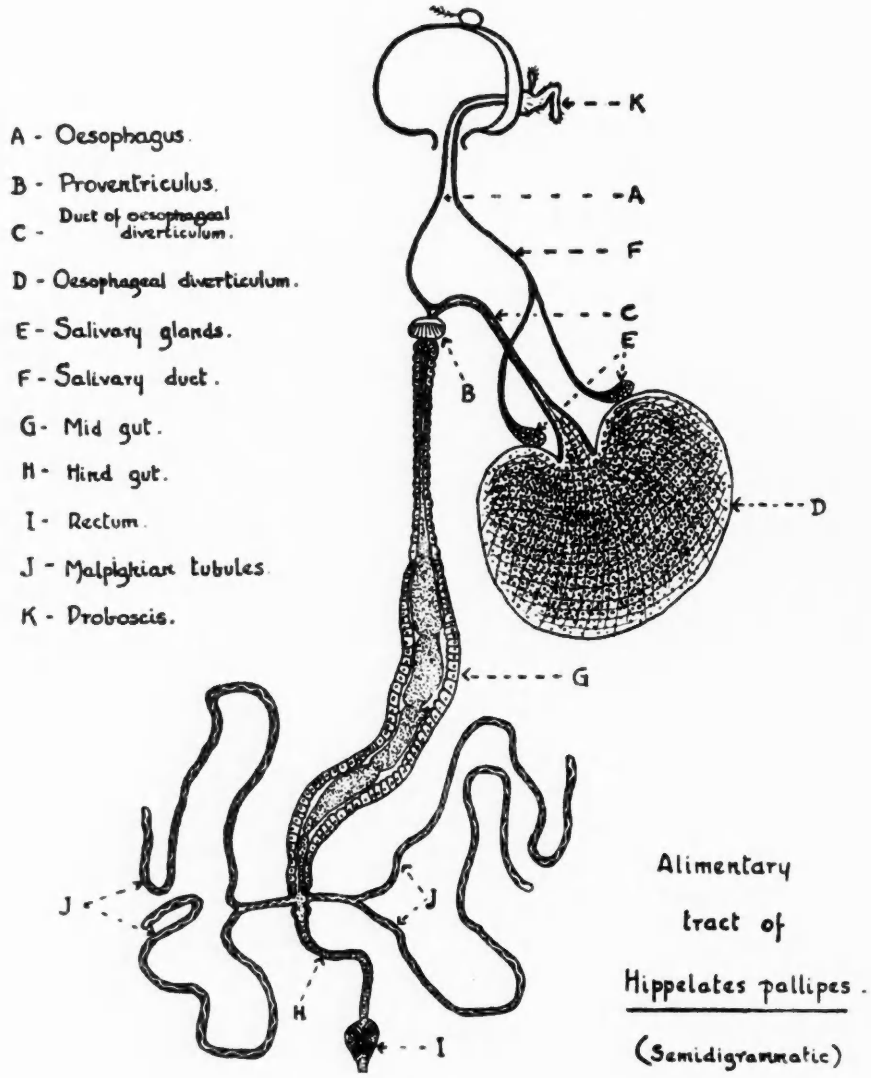

FIG. 1.

The best way to describe the anatomy of the digestive system of *Hippelates pallipes* will be by reference to the accompanying diagrams. Text-fig. 1 shows more or less the appearance of the organs as they are pulled out on to a slide in the dissecting microscope, except that the oesophagus and the common salivary duct have been included for completeness. Actually, these last two are almost

never met with in the ordinary routine dissection of flies, because they are so very fine and delicate that they are practically always torn off. Text-fig. 2 shows the relative position of the various organs *in situ* in the fly. The two bulges in the ventral abdominal wall indicated in this diagram are very commonly seen. The first bulge when present is caused by the distended oesophageal diverticulum full of blood, pus or serum. The second bulge is usually due to the ovaries. When these are large it is correspondingly prominent.

The oesophagus, as mentioned previously, is very thin and delicate, even

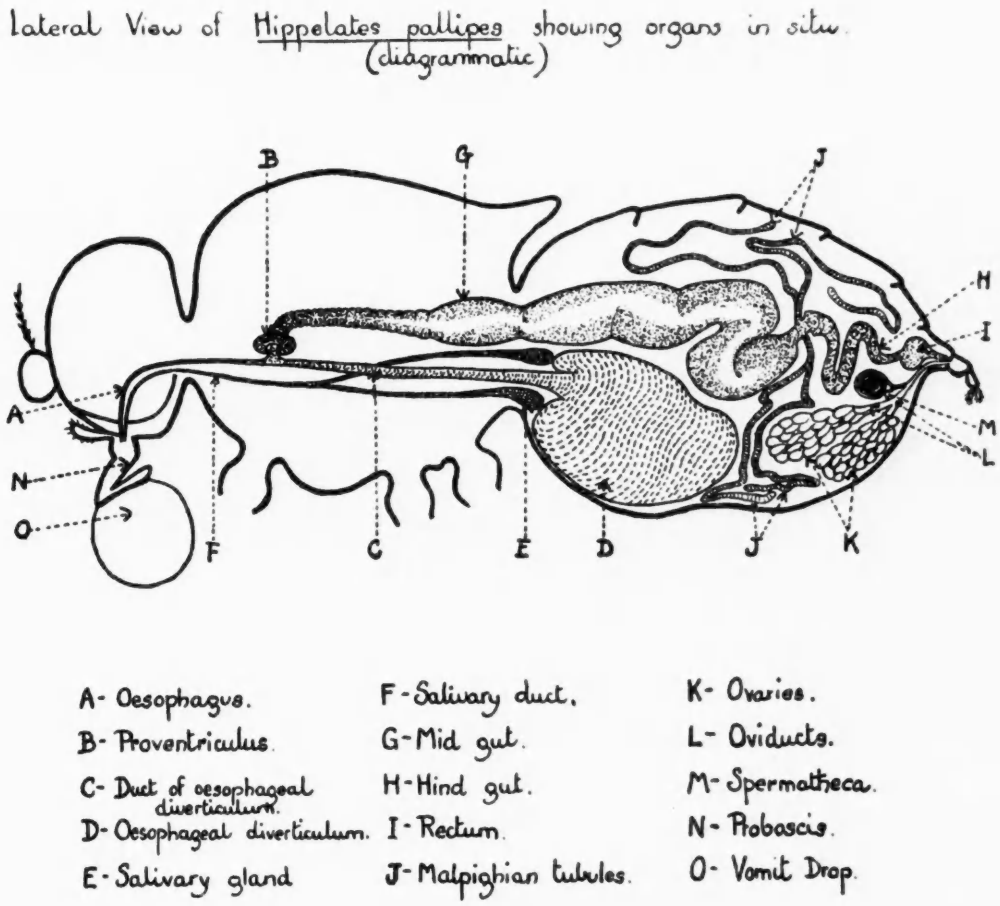

FIG. 2.

finer perhaps than indicated in the diagrams. It runs from the proboscis to the proventriculus, where it meets the mid gut and the duct of the diverticulum. When one realizes its extremely tenuous nature, it is easy to comprehend why the oesophagus is almost invariably broken or torn off when the other organs are removed from the fly. The course of the oesophagus in the fly is almost continuous with that of the duct of the diverticulum. The latter begins with a diameter of about two or three times that of the oesophagus, and gradually enlarges until it joins with the diverticulum itself.

The oesophageal diverticulum or crop is frequently the largest organ in the abdominal cavity of the fly. It is capable of great and rapid distention when serum or blood is quickly ingested while the fly is feeding. Then the diverticulum slowly empties itself again by means of the regurgitation process which produces the ' vomit drop,' as the ingested material goes over into the mid gut. The ventral oesophageal diverticulum is a thin-walled structure and is supplied with but few muscle fibres compared to the mid gut and hind gut.

The proventriculus or gizzard has quite a characteristic and readily recognizable appearance. It lies at the junction of the oesophagus, the duct of the diverticulum and the mid gut. The mid gut itself is a highly muscular organ, though capable of considerable distention in the central and posterior portions. The rings of strong circular muscle fibres at the anterior end are very pronounced, especially in unfed insects. About two-thirds or three-quarters of the way along its course the mid gut is bent, and a loop projects forward when the organ is lying *in situ* in the abdominal cavity of the fly. This S-shaped loop is invariably present. The mid gut ends at the junction of the malpighian tubules with the intestine.

There are four malpighian tubules in *Hippelates pallipes*. These come off by two common ducts, each of which later subdivides into two tubules, making four in all. The yellow colour and general appearance of the malpighian tubules in a fresh dissection is unmistakable. The terminal portions of the malpighian tubules are often distended with air and white in colour.

The hind gut is relatively short and extends from the junction of the malpighian tubules with the intestinal tract to the rectum. The greatest diameter of the hind gut is just posterior to the malpighian tubules, from which zone the diameter rapidly tapers off to that of a thin channel. Circular bands of muscle fibres are fairly apparent but not so striking as those at the beginning of the mid gut. The rectum is pear-shaped and contains four rectal papillae.

The ovaries are white in the fresh specimen, and vary much in size according to the stage of development of the ova. However, they can readily be distinguished from the organs of the digestive system, as can the spermatheca with its two ducts coiled up like a watch-spring. In the male the testicles are light brown or yellowish and quite distinctive from everything else.

The position of the salivary glands is unlike that seen in house-flies or mosquitoes. The glands are always attached loosely to the anterior surface of the diverticulum of the oesophagus. A microphotograph (Plate IV, fig. 1) has been taken to show this, and indicates quite clearly the bulbous lobes of the salivary glands lying on the surface of the oesophageal diverticulum, though apparently only attached very superficially. In the microphotograph the duct of the diverticulum comes off between the two salivary glands.

The salivary ducts from each gland are large in diameter, though not quite as big as the diameter of the duct of the diverticulum. The diameter of the ducts of the two salivary glands remains large until they join together to form

the common salivary duct. The latter is much finer, even thinner than the oesophagus, so that in cases where the oesophagus is removed without breaking the common salivary duct is almost invariably ruptured. Indeed, we have only once been able to remove the common salivary duct intact. It is thus obvious that, even if the head of the fly is not cut off before removing the organs from the abdominal cavity, it is not very difficult by gentle traction to rupture the oesophagus and common salivary duct and to pull out the rest of the organs in a single mass.

PHYSIOLOGY OF SOME OF THE DIGESTIVE PROCESSES

Graham-Smith (1913) has made a very careful study of the digestive mechanism of the common house-fly, *Musca domestica*. Most of the results reported in the following paragraphs were obtained from experiments made in a manner similar to those of that author. It has been of great interest to learn how closely many of the processes that occur in *Hippelates pallipes* simulate those which Graham-Smith and others had observed previously in *Musca domestica*.

Graham-Smith fed house-flies on carmine gelatin and observed the rate at which such coloured substances passed into the mid intestine from the crop, and also the length of time that they remained in the mid gut. We fed *Hippelates pallipes* on blood serum stained with brilliant cresyl blue, but did not obtain very satisfactory results. If the dye was present in a high concentration, the flies would not feed on the serum, and if the serum was only faintly tinged with the dye it did not show up well in the intestinal tract of the fly. However, it proved unnecessary to feed *Hippelates* on serum stained with synthetic dyes, because haemoglobin served the purpose just as well, if not better. *H. pallipes* readily ingest blood-tinged serum or even pure blood from ulcers, and no difficulty was experienced in the laboratory in feeding them on a blood clot spread out on a piece of filter paper in the cover of a petri dish.

The flies were fed in individual tubes, and the time at which each fly took a meal of blood was noted with a wax pencil on the outside of the glass tube. The flies were subsequently dissected at different intervals following the feed on the blood clot, to obtain an idea of the rapidity with which the food passed through the fly's digestive tract. The tubes with fed flies were left on the desk in the laboratory, so that they were under more or less natural conditions as regards temperature.

Hippelates pallipes will not live much longer than 24 hours in a test-tube plugged with dry cotton-wool. It was found, however, that in petri dishes with a few grains of ordinary sugar on the bottom the flies would live longer than in cages. So dissections which were made two and three days after the original meal on the blood clot were on flies which had been kept in the laboratory desk in petri dishes with a little sugar. The sugar appeared to absorb enough moisture to satisfy the water requirements of the flies.

Groups of twelve *Hippelates pallipes* each were dissected at 1, 5, 10, 15, 20 and 30 minute intervals after they had fed on the blood clot. The position of the blood column in the mid gut and hind gut was noted for each fly, as well as the amount of blood in the crop and in the rectum. Other groups of twelve flies each were dissected after intervals of 1, 3, 6, 12, 24, 48 and 72 hours, and similar observations were made in regard to the position of the blood meal in their alimentary tracts. The findings from this study were briefly as follows.

As the fly took in its meal of blood and serum, most of the ingested material went straight into the diverticulum of the oesophagus. However, a small amount went directly into the mid gut as well. In one fly dissected one minute after the meal, a thin column of blood extended a fifth of the way down the mid gut from the proventriculus, and in another the blood column had already reached to the level of the malpighian tubules. On the average, it extended about half the length of the mid gut in this space of time. After a 5-minute interval the mid gut was usually about two-thirds full while the hind gut and rectum were still empty. At this time the blood in the crop and in the mid gut was still red and showed no evidence of chemical change due to action of the digestive juices of the fly.

After an interval of 10 minutes from the initial feed, the column of blood had reached the posterior end of the mid gut in all but one fly, and in a single insect it had already begun to enter the hind gut. However, although the thin column of blood was rapidly entering the fly's stomach, the major portion was still in the diverticulum of the oesophagus 10 minutes after the fly fed on the blood clot.

At 15 and 20 minutes after the blood meal, the position of the food in the intestinal tract of the fly was essentially the same as that after 10 minutes. But after a 30-minute interval the column of blood had extended to the end of the mid gut in all the twelve flies dissected, and in three it had already entered some distance into the hind gut.

In *Hippelates pallipes* dissected 1, 3 and 6 hours after the initial feed, both the oesophageal diverticulum and the mid gut were well distended with blood, and the condition of the hind gut varied from empty to completely full, with averages of one-quarter full at one hour, and one-half full at six hours. At the 3-hour period it was noticeable that the tips of the proboscis of many of the flies were stained red with blood, indicating that the regurgitation process had been going on. Six hours after feeding, the flies appeared to have about as much blood in the oesophageal diverticulum as in the stomach. At 12 hours, on the other hand, there was more blood in the mid gut than in the crop. In the mid gut the blood was becoming black, while the colour of the contents of the oesophageal diverticulum approached carmine. After the 12-hour interval, half the flies had altered blood in the rectum as well as in the hind gut.

By 24 hours or one day following the original meal on the blood clot, the character of the ingested material was altered throughout the alimentary tract

of the flies. In the mid gut, hind gut and rectum the blood was black or dark brown, while that in the diverticulum of the oesophagus was pale pink and much diluted with water. At this time the column of altered blood extended the entire length of the hind gut. In some cases the latter organ was much distended, and in others the blood column consisted merely of a thin brown streak in the centre of the intestinal canal. The rectum also contained some altered blood, either in large or small amount.

On the 2nd and 3rd days the crop was largely empty, and what blood still remained in it was much diluted with water and pale brown in colour. The mid gut also was fast loosing its contents. In 2 of the 12 flies dissected after a 3-day interval the mid gut was entirely empty, and in 7 the oesophageal diverticulum was devoid of blood. Even in the rectum and hind gut the black residue of the blood meal was being evacuated, so that usually only a thin streak remained in the centre of the canal of the hind gut, and a small portion of brownish material in a half or a third of the rectum. The results from this series of dissections have been tabulated and are given below :—

TABLE I

Summary of results of dissection of *Hippelates pallipes* fed on blood clots, as regards the position of the blood meal in the intestinal tracts of the flies

Time since flies fed on blood clot	No. of flies dissected	No. of flies with oesophageal diverticulum full of blood	No. of flies with the column of blood extending the entire length of the mid gut	No. of flies with the column of blood extending the entire length of the hind gut	No. of flies with the rectum full of blood
1 minute	12	12	1	—	—
5 minutes	12	12	3	—	—
10 ,,	12	12	11	—	—
15 ,,	12	12	10	—	—
20 ,,	12	12	10	—	—
30 ,,	12	12	12	—	—
1 hour	12	12	12	—	—
3 hours	12	12	12	2	1
6 ,,	12	12	12	1	—
12 ,,	12	11	12	6	6
1 day	12	7	12	12	10
2 days	12	—	5	12	9
3 ,,	12	—	—	6	3

TABLE II

Approximate position of the ingested material in the alimentary tract of *Hippelates pallipes*, at various time intervals after a meal on a blood clot

Time since flies fed on the blood clot	Condition of the oesophageal diverticulum	Condition of the mid gut	Condition of the hind gut	Condition of the rectum
1 minute	Fully distended	Column of blood about half way down	Empty	Empty
5 minutes	,, ,,	Column of blood about two-thirds down	,,	,,
10 ,,	,, ,,	Practically full	,,	,,
15 ,,	,, ,,	,, ,,	,,	,,
20 ,,	,, ,,	,, ,,	Practically empty	,,
30 ,,	,, ,,	Fully distended	About one-quarter full	,,
1 hour	,, ,,	,, ,,	About one-quarter full	,,
3 hours	,, ,,	,, ,,	About one-third full	,,
6 ,,	,, ,,	,, ,,	About half full	,,
12 ,,	Not entirely full	,, ,,	About two-thirds full	About half full
1 day	About nine-tenths full	,, ,,	Fully distended	Fully distended
2 days	About half full or less	About three-fourths full	,, ,,	About three-fourths full
3 ,,	About one-tenth full	About one-fourth full	About two-thirds full	About one-third full

The observation that at 6 hours after a meal on a blood clot *Hippelates pallipes* appeared to have about as much blood in the crop as in the stomach, whereas at 12 hours there was more of the ingested material in the mid gut than in the oesophageal diverticulum, fits in well with the results of previous work. Kumm, Turner and Peat (1935), in a study of the duration of motility of the spirochaetes of framboesia tropica in these flies, showed that the majority of the ingested treponemes were found in the oesophageal diverticulum for the first 8 hours following the infecting feed, after which time they had passed over into the stomach.

In the dissections of *H. pallipes* to determine the position of the ingested material at various time intervals after the flies had fed on a blood clot, it was found that at the 3-hour period the tips of the probosces of many of the flies were stained red with fresh blood, indicating that a process of regurgitation had been going on. This mechanism, which produces the so-called ' vomit drop,' is probably very important in the transmission of disease by these flies, and so merits a careful description. Graham-Smith (1913), in discussing the common house-fly, wrote that the regurgitation of food from the crop or oesophageal diverticulum of the fly in the form of a vomit drop was one of the most important methods by which it infected the human body, as well as food, with pathogenic organisms.

We have observed the mechanism by which the ' vomit drop ' is produced in *Hippelates pallipes* on repeated occasions by the following method. A fly is allowed to feed to repletion on an infectious yaws lesion, or indeed on any ulcer which is secreting serum or pus in profusion, and then the insect is observed in a petri dish or large bore test-tube. The fly will crawl round on the inside of the tube or dish and occasionally come to rest for brief periods of time. After half an hour or usually less, when the fly keeps still enough to make it possible to focus the dissecting microscope on her, one can see the ' vomit drop ' appear just as has been described in the case of *Musca domestica.* The insect first expels some fluid from her oesophageal diverticulum and the spherical ' vomit drop ' can be seen swelling up on the end of her proboscis.

If the fly is observed standing upside down on the lid of the petri dish, it is easy to get the tip of her proboscis in good focus in the microscope. Then one can observe the ' vomit drop ' being slowly re-swallowed apparently into the mid gut. Soon regurgitation begins again and the sperical drop increases or decreases in size according as the fluid is expelled or withdrawn rhythmically. The process is repeated over and over again many times.

A microphotograph has been taken of the head of a *Hippelates pallipes* in the act of regurgitation with the ' vomit drop ' in sharp focus (Plate IV, fig. 2). The attachment of the ' vomit drop ' to the tip of the proboscis is not very clear, because it was not possible to get the proboscis in the same focal plane as the ' vomit drop.' But, if it is remembered that the proboscis of the fly is in three segments, one can distinguish fairly readily how the ' vomit drop ' is attached

to the distal segment. This method of attachment is also indicated in text-fig. 2.

By analogy with the regurgitation process which occurs in the house-fly, apparently *Hippelates pallipes* swallow their food first into the oesophageal diverticulum. This enables them to ingest a large amount in a short time, so that they can obtain the requisite food before being brushed off the wound or ulcer by the patient. Then the flies slowly regurgitate ' vomit drops ' and later re-swallow the fluids ingested by them into their stomachs. A semi-coagulated ' vomit drop ' on the tip of the proboscis is not uncommonly seen in dead flies which fed well shortly prior to their death. On occasions these ' vomit drops ' will act as glue and stick the flies to the glass sides of the catching tube or stick two flies together. Flies often regurgitate a ' vomit drop ' just before or after death. This may be very important in the case of infected *Hippelates pallipes* which die stuck to the drying serum on the surface of non-specific ulcers or wounds.

' Vomit drops ' from flies which fed to repletion on infectious lesions of framboesia tropica have been examined. In one fly, 4 motile *T. pertenue* were demonstrated in the ' vomit drop,' 4 more in the oesophageal diverticulum and 6 in the stomach of the fly. In another infected insect, part of the contents of the crop was deliberately expressed just after death by pressure with the dissecting needle on the ventral abdominal wall, and 30 *Treponema pertenue*, 28 of them motile, were found in this artificial ' vomit drop.' A third specimen of *Hippelates pallipes* was observed in the microscope after filling itself so full of serum from an infectious case of yaws that it could hardly crawl about. It stayed in one place slowly regurgitating and re-swallowing its ' vomit drop.' After a while it inadvertently let the ' vomit drop ' touch the side of the glass tube and a minute droplet separated off and adhered to the glass. The fly was quickly removed to another container and this ' vomit spot ' emulsified in normal saline and examined in the dark-field microscope. One non-motile *Treponema pertenue* was seen.

The actual number of ' fly specks ' deposited by *Hippelates* flies after feeding is of considerable importance. With the idea of obtaining more accurate information on this point, the following experiment was performed. One hundred *Hippelates* flies were caught at Mount Pleasant, in the parish of St. Andrew, after they had fed to repletion on an ulcer on the foot of one of the patients. Actually the ulcer was not an infectious framboesial lesion, but that made no difference, for the object of the experiment was simply to obtain information on the number of the ' fly specks ' deposited and not to discover whether these were infected with yaws spirochaetes or not. Each fly was caught in a clean glass tube and the number of ' fly specks ' deposited by it every hour was recorded.

A ' fly speck ' on the wall of the test-tube might be either a droplet from a ' vomit drop ' or else a ' faecal spot.' As we were not sure that we could distinguish ' vomit spots ' from ' faecal spots,' it seemed best to count all of them

and to classify the total figure as ‘ fly specks.’ The number of ‘ fly specks ’ deposited by each fly each hour was counted up to 6 hours after the initial feed. A final observation was also made after 24 hours, and then the average number of ‘ fly specks ’ deposited each hour was calculated as per the following Table and graph (text-fig. 3) :—

TABLE III

Period of time following initial feed on ulcer on patient	No. of ‘ fly specks ’ deposited during this period	No. of flies alive during this period	Average no. of ‘ fly specks ’ per fly deposited during this period of time
0– 1 hours	155	100	1·55
1– 2 „	420	100	4·20
2– 3 „	441	100	4·41
3– 4 „	184	100	1·84
4– 5 „	185	94	1·97
5– 6 „	152	86	1·77
6–24 „	1,216	31	39·23

To be compared with the other figures based on periods of one hour only, the 6–24 hour total should be divided by 18, as this is an 18-hour period. The result is 2·18, which is the figure used in the graph.

Number of " vomit drops and faecal spots " deposited by Hippelates flies during various intervals of time after feeding on an ulcer.

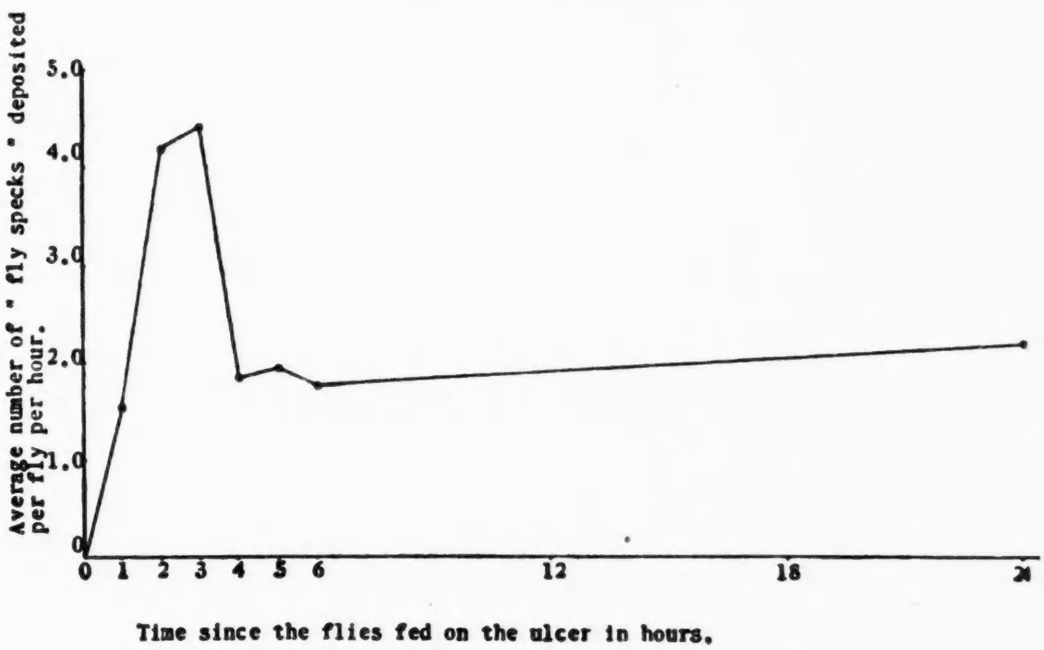

FIG. 3.

There was considerable variation in individual flies as regards the number of ' fly specks ' deposited ; so for this reason it was deemed necessary to calculate the average figures on not less than 100 insects. In the first hour after feeding the number of ' fly specks ' varied from 0 to 10, and in the second hourly period from 0 to 16. In the 18-hour period, from 6 to 24 hours after the original meal on the ulcer, the number of deposits varied in individual *Hippelates* from a minimum of 8 to a maximum of 69 ' vomit and faecal spots.'

Adding up the average numbers of ' fly specks ' per fly per hour for the 24 hours gives a total of 54·97, indicating that an ordinary healthy *Hippelates* fly deposits about 55 ' vomit and faecal spots ' in the first 24 hours after feeding on an ulcer. This gives a mean of 2·3 ' fly specks ' per hour during these 24 hours. However, the most dangerous time from the standpoint of the potential transmission of disease would be at the peak of the curve, namely 2 or 3 hours after feeding. When it is recalled that approximately two-thirds of the *Treponema pertenue* ingested from an infectious yaws lesion are still actively motile in the fly's oesophageal diverticulum at this time, it is not difficult to conceive how yaws might be transmitted.

In order to interpret more accurately the significance of this last experiment, it would be of value to be able to distinguish ' vomit spots ' from ' faecal spots,' and to know if the flies deposited more of the former than of the latter. Since *Treponema pertenue* live for at least 7 hours in the oesophageal diverticulum, they could obviously be regurgitated in an infectious state in ' vomit drops.' Whereas, since they lose their motility so quickly in the stomach, they would probably all be dead before they left the intestinal tract of the fly in the form of faeces.

Three *Hippelates pallipes* were fed on a blood clot and observed continuously for 4 hours in an attempt to gain further light on this point. Each fly was isolated in an individual tube, and each time that it regurgitated a ' vomit drop ' or deposited a ' vomit spot ' a note was made. Two of the three flies had started to expel and retract ' vomit drops ' within 5 minutes of their original meal on the blood clot. The third did not begin this process until 15 minutes had elapsed. The regurgitation and re-swallowing of blood was often repeated many times before the drop would touch the wall of the glass tube and a minute droplet become separated off.

One fly regurgitated 10 times in rapid succession, three-quarters of an hour after its blood meal, and another insect repeated this process 13 times without stopping after a 56-minute interval had elapsed. As a rule there was a pause of some minutes between each time a fly regurgitated. However, when the flies regurgitated several times in rapid succession, the actual process of regurgitation and re-swallowing of the blood lasted from 10 to 15 seconds, though sometimes longer. Occasionally the flies would crawl round with a ' vomit drop ' on the end of their probosces. During the whole of the first hour after feeding on the blood clot, one fly expelled and retracted a ' vomit

drop ' 17 times, another insect 26 times, and a third one 48 times. The average for the first hour was just over 30.

Most of the regurgitation and re-swallowing took place within the first hour after the blood meal, whereas the depositing of ' vomit spots ' on the wall of the glass tube did not reach its peak till the third hourly period. No faecal spots were deposited during the first 4 hours after feeding, in as far as we could see. This does not fit in with Graham-Smith's results on *Musca domestica*, but is in line with what we should have expected from the results of dissections of flies fed on blood clots. Since in that series of dissections blood was found in the rectum fairly consistently after the 12-hour interval, one wonders if the majority of the ' fly specks ' deposited in the 6–24 hour period after a meal were not ' faecal ' rather than ' vomit spots.' The average results for three *Hippelates pallipes* are tabulated below :—

Table IV

Interval of time since fly fed on blood clot	Average no. of ' vomit drops ' expelled and withdrawn per fly	Average no. of ' vomit spots ' deposited on the wall of the glass tube	Average no. of ' faecal spots ' deposited on the wall of the glass tube
First hour	30·3	2·7	0
Second „	3	8	0
Third „	4	10	0
Fourth „	0	9	0

These three *Hippelates pallipes* averaged more ' fly specks ' per fly per hour than those reported in the first series of 100 flies. There may be several explanations of this apparent irregularity : first, the sample was very small ; second, the flies were fed on blood instead of serum from an ulcer ; third, the flies were watched continuously and so very minute droplets, that might otherwise have been missed, were recorded ; fourth, the glass tubes in the first experiment were laid on the laboratory bench and hence at normal room temperature. In the second case, however, the insects were watched with a powerful light shining on them to facilitate observation. Both this light itself and the heat that radiated from it may have tended to increase the activity of the flies, and may have caused them to regurgitate more frequently than they would normally have done.

SUMMARY

1. In order to investigate what happens to *Treponema pertenue* after ingestion by *Hippelates pallipes*, and also the potentialities of these flies for the transmission of yaws, it is essential to have a clear understanding of the anatomy and physiology of the digestive system of the insects. The studies reported in this paper were made with that object in view.

2. Some of the feeding habits of *Hippelates pallipes* are discussed. Microphotographs are given of fed and unfed flies.

3. The anatomy of the digestive system, exclusive of the mouth-parts, is described, together with two explanatory diagrams.

4. The results of a series of dissections of *Hippelates pallipes* fed on a blood clot are given, with special reference to the position of the blood meal in the alimentary canal of the flies at various intervals of time after the initial feeding.

5. The mechanism of regurgitation of food from the crop or oesophageal diverticulum of the fly is described and its significance discussed. A microphotograph of a *Hippelates pallipes* in the act of regurgitating a ' vomit drop ' is included.

6. *Hippelates* flies deposit about 55 ' fly specks ' in the first 24 hours after feeding on an ulcer. The maximum number of ' fly specks ' per fly per hour are deposited in the periods, two and three hours after the meal.

7. The ' fly specks ' deposited during the first four hours after a meal on a blood clot are ' vomit spots ' rather than ' faecal spots.' During the first hour after feeding many more ' vomit drops ' are regurgitated than ' vomit spots ' deposited.

ACKNOWLEDGMENTS. It is a pleasure to acknowledge the help and advice received during the course of this study from Dr. Thomas B. Turner, Director of the Jamaica Yaws Commission. For the microphotographs of the flies, the writer is greatly indebted to the exceptional skill and care of Mr. Denis M. Gick, of Kingston.

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PLATE III

EXPLANATION OF PLATE III

Fig. 1. Unfed female *H. pallipes* in lateral view, showing general characters.

Fig. 2. Fully fed female *H. pallipes*, showing enormous distension of abdomen.

PLATE III

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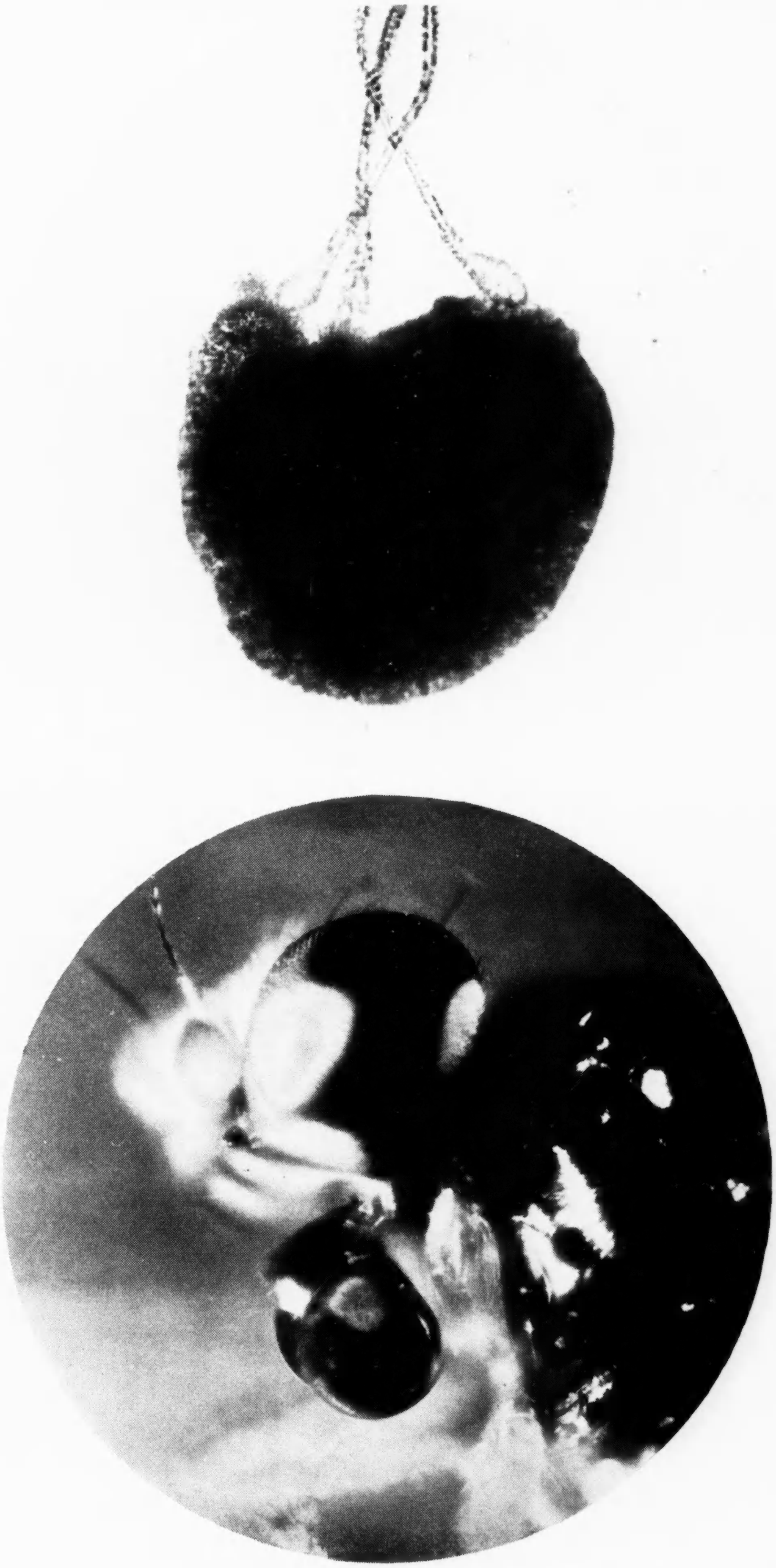
EXPLANATION OF PLATE IV

Fig. 1. Oesophageal diverticulum of *H. pallipes*, showing attachment of salivary glands.

Fig. 2. Head of *H. pallipes*, showing ' vomit drop ' at end of proboscis.

PLATE IV



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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS GLOSSINA WIEDEMANN BASED ON A COMPARATIVE STUDY OF THE MALE AND FEMALE TERMINALIA

(*Continued from vol.* 28, *page* 588)

BY

W. S. PATTON

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

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In continuation of this series, I propose in this part to describe shortly and to illustrate the segmentation of the abdomen and terminalia of *Hypoderma* Latr. and *Gasterophilus* Leach as exemplified by those of *bovis* L. and *intestinalis* de Geer respectively.

SHORT DESCRIPTION OF THE SEGMENTATION OF THE ABDOMEN AND OF THE MALE AND FEMALE TERMINALIA OF *HYPODERMA* AS EXEMPLIFIED BY THOSE OF *BOVIS* L.

Hypoderma bovis L. SEGMENTATION OF MALE ABDOMEN. SCLERITES. The ventral view of the ♂ abdomen of *bovis* showing the segmentation and sclerites is illustrated in fig. 1, *a* ; as the sclerites are all labelled, it is only necessary to describe in particular those at the end of the abdomen which are associated with the terminalia. It should be noted, however, that tergum 1 is distinctly separated ventrally from tergum 2, and that spiracle 1 is situated in the membrane close to the latero-ventral edge of the tergum. Spiracles 2, 3, 4 and 5 are located close to the latero-ventral edges of their respective terga. Sterna 2,3 and 4 are large and densely covered with hairs. Sternum 5 (fig. 1, *a*) is a large lobed plate. Tergum 6 has possibly fused with tergum 7, and is represented by the triangular-shaped portion at the posterior edge of the latter. Sternum 6 (or an apodeme) is a strong flat rod nearly reaching to the antero-lateral edge of tergum 7 on the right (fig. 1, *b*) ; it has a deep ventral and a large narrow dorsal flange on the left. Tergum 7 is somewhat asymmetrical (fig. 1, *b*), and is a rounded plate forming with tergum 10 the end of the abdomen ; its posterior portion may possibly represent tergum 6. Segment 8 is wanting.

SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. SCLERITES. Figs. 2; 3. The ninth tergo-sternum is a large convex plate, the posterior processes almost meeting. Tergum 10 is a small, wide, rounded plate, the postero-dorsal border slightly turned downwards ; its anterior border is deeply incised and is closed anteriorly by the diverging posterior portions of the fused anal cerci, the incision being bridged by a membrane on which the anal opening lies. Dorso-laterally the tergum extends into a long, broad, bent, flat plate which

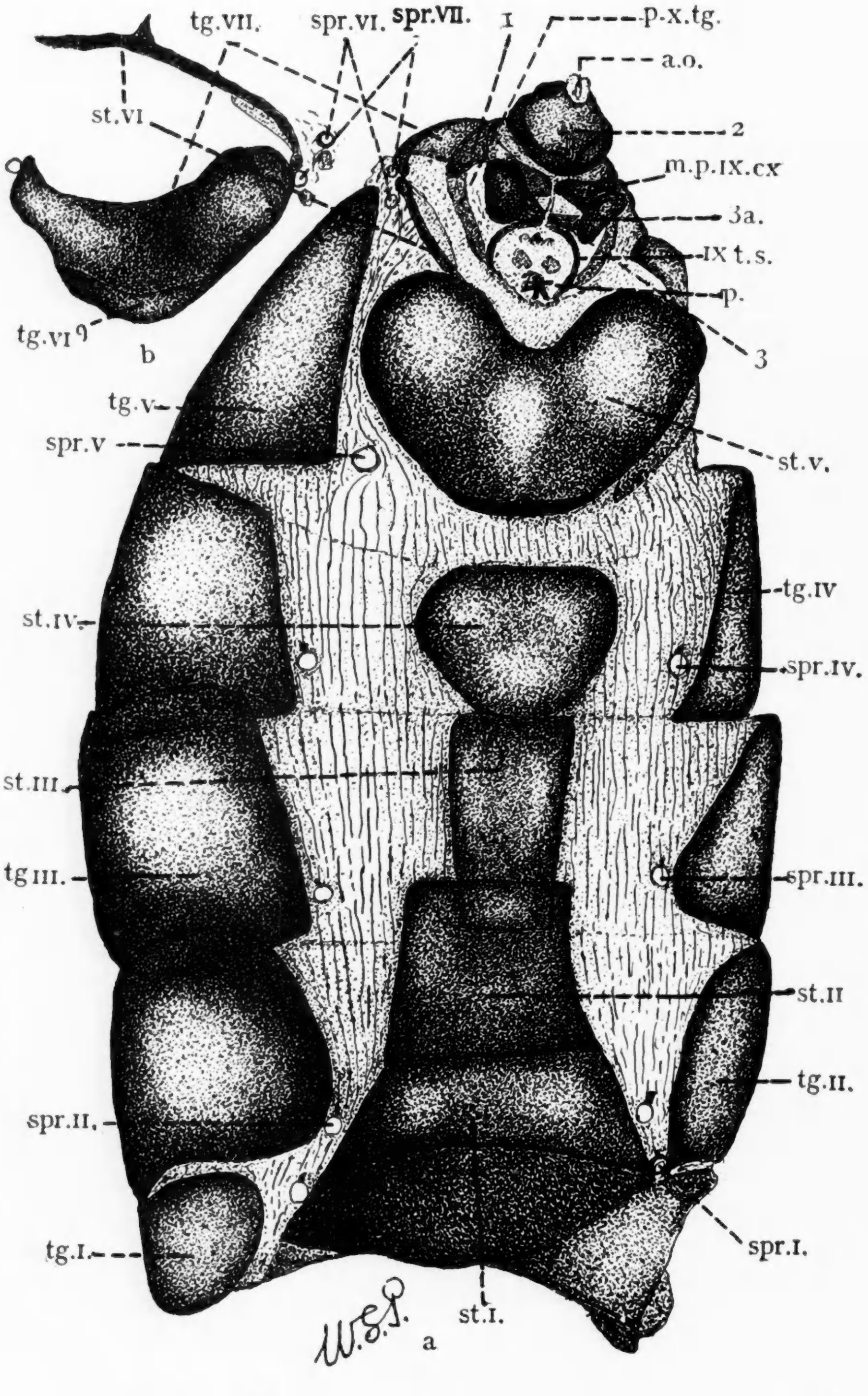

FIG. 1. *a.*—Ventral view of ♂ abdomen of *H. bovis* showing segmentation and sclerites; *b.*—Ventral view of tergum 7 and sternum 6; 1.—Tenth tergum; 2.—Anal cerci; 3.—Proximal segment of ninth coxite; 3*a.*—Distal segment of same; *p.*—Phallosome; *a.o.*—Anal opening; *ix.t.s.*—Ninth tergo-sternum; *p.x.tg.*—Process of tergum 10 articulating with ninth tergo-sternum; *m.p. ix.cx.*—Median process of ninth coxite; *spr. i, spr. ii, spr. iii, spr. iv, spr. v, spr. vi, spr. vii.*—Spiracles 1–7 inclusive; *st. i, st. ii, st. iii, st. iv, st. v, st. vi.*—Sternites 1–6 inclusive; *tg. i, tg. ii, tg. iii, tg. iv, tg. v, tg. vi, tg. vii.*—Terga 1–7 inclusive.

articulates with the side of the ninth tergo-sternum. The antero-lateral edge of the tergum is continuous with the outer wall of the distal segment of the ninth coxite (fig. 2).

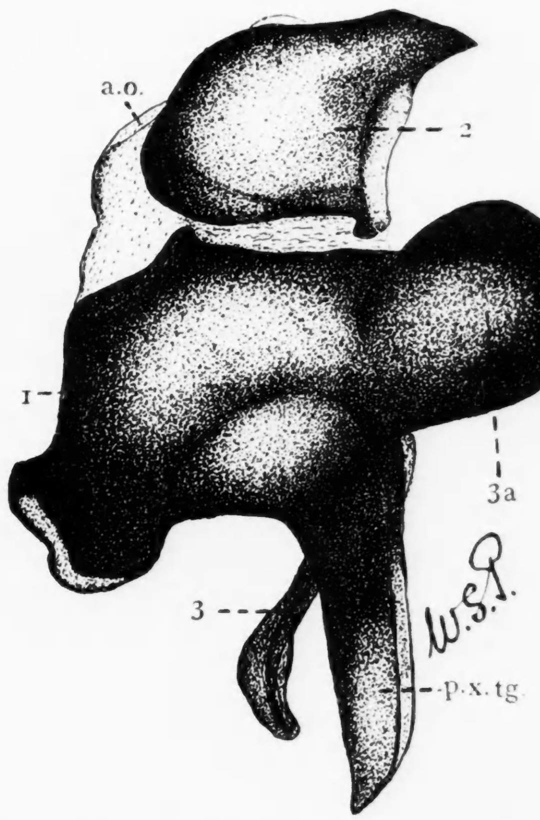

FIG. 2. Tenth tergum, anal cerci and ninth coxite of *bovis* in side view ; lettering as in fig. 1.

APPENDAGES. *Ninth Coxite.* Lateral view. *Distal Segment.* Fig. 2. The distal segment is a short, bluntly rounded plate shaped like the human hand (without the fingers), and its rounded end is turned inwards towards its fellow. Each lies some distance above and on each side of the corresponding anal cercus, and, as noted above, the outer wall of each is continuous with that of tergum 10. A median process from each (fig. 1, *a*) extends across towards the middle line. *Proximal Segment.* Lateral view. Fig. 2. The proximal segment is continuous with the inner dorsal wall of the distal segment, and is a broad flat plate which articulates with its fellow (to which it is joined by membrane) against the posterior processes of the ninth tergo-sternum. *Anal Cercus.* Lateral view. Fig. 2. The two anal cerci are fused anteriorly to form a pointed beak-like process (in side view) ; posteriorly they are separated, forming rounded plates closing the incision of tergum 10.

PHALLOSOME. Lateral view. Fig. 3, *a.* The phallosome is long, the proximal part of the body consisting of a long tube, continuous with which ventrally are the two long flat struts expanded at their ends and bent upwards

and backwards in hooks (fig. 3); the remainder of the phallosome is membranous, the membrane extending beyond the struts and bearing the opening of the ejaculatory duct; between the ends of the struts the membrane is

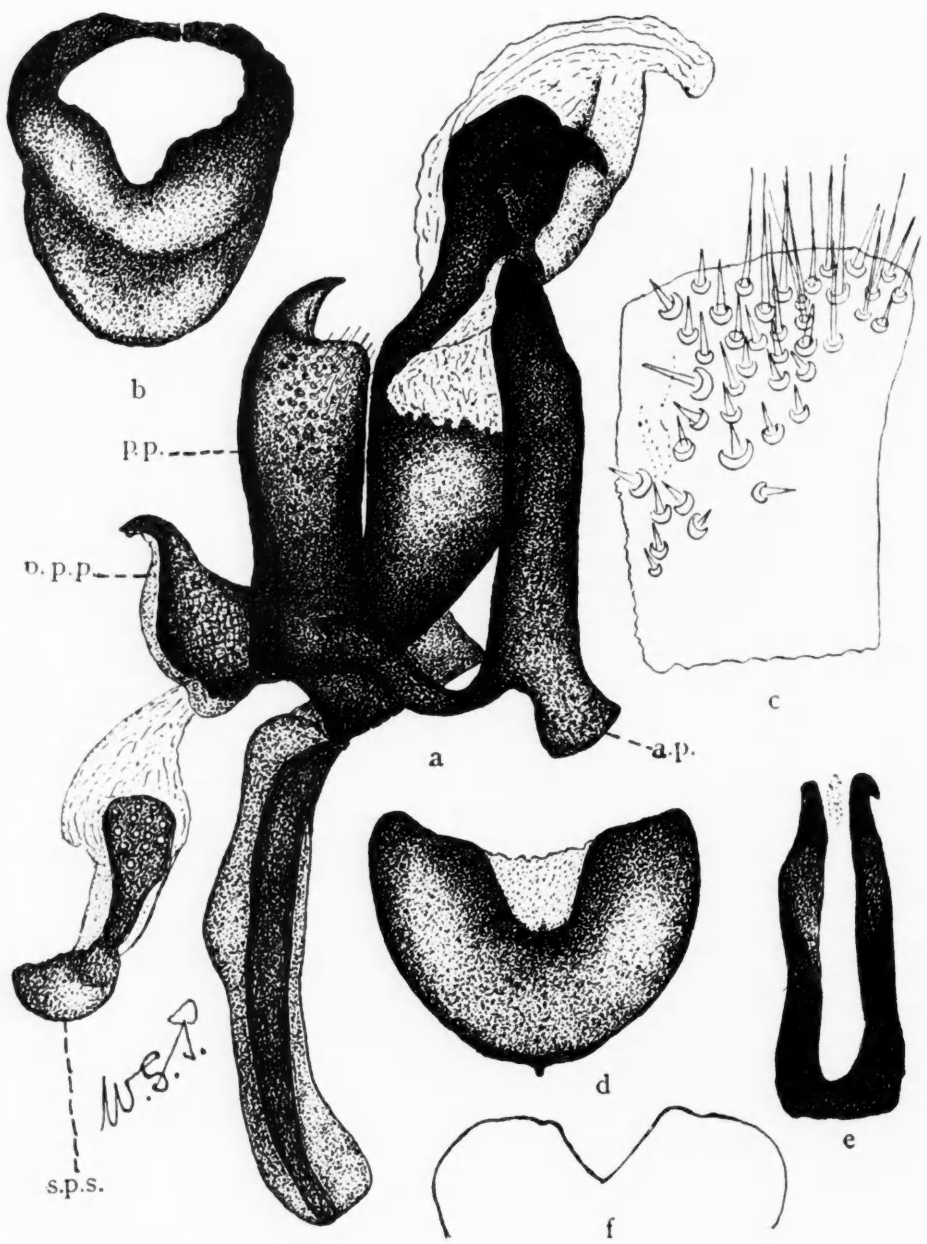

FIG. 3. *a.*—Phallosome, sperm pump sclerite (*s.p.s.*), apodeme, posterior process of phallosome (*p.p.p.*), and one paramere of *bovis* in side view; *a.p.*—anterior part of paramere; *p.p.*—posterior part of same; *b.*—Ninth tergo-sternum; *c.*—Sensory spines from posterior paramere; *d.*—Fifth sternum; *e.*—Dorsal view of struts; *f.*—Outline of anterior end of fifth sternum showing different structure.

chitinized. In *H.* (*Oedemagena*) *tarandi* L., which is closely related to *bovis*, this chitinization is better developed. *Posterior Process of Phallosome.* Fig. 3. Wide and saddle-shaped, the end bent. *Sperm Pump Sclerite.* Fig. 3. Long. *Apodeme of Phallosome.* Fig. 3. Long and broad. *Parameres.* Lateral view.

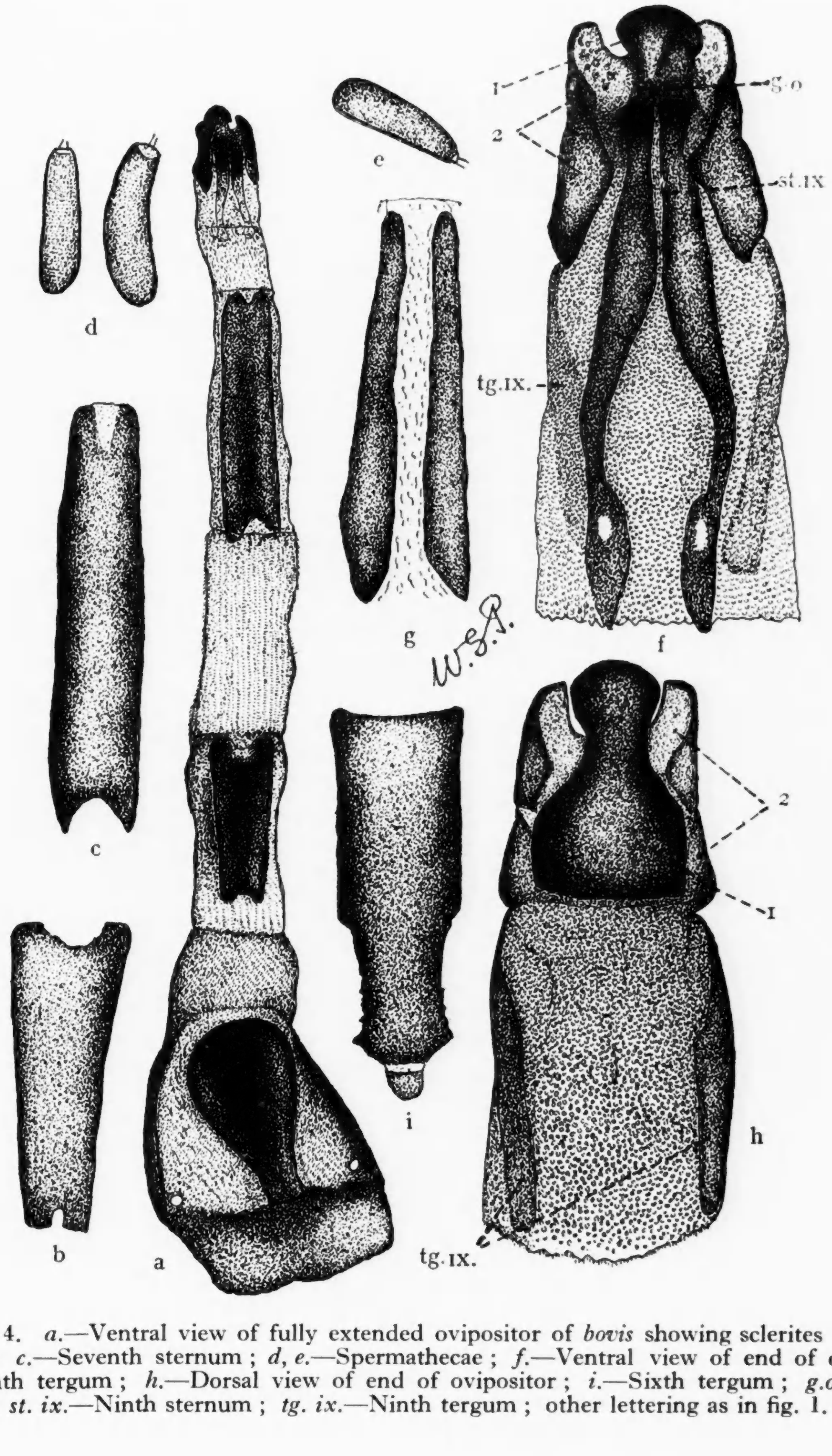

FIG. 4. *a.*—Ventral view of fully extended ovipositor of *bovis* showing sclerites *b.*—Sixth sternum ; *c.*—Seventh sternum ; *d, e.*—Spermathecae ; *f.*—Ventral view of end of ovipositor ; *g.*—Seventh tergum ; *h.*—Dorsal view of end of ovipositor ; *i.*—Sixth tergum ; *g.o.*—Genital opening ; *st. ix.*—Ninth sternum ; *tg. ix.*—Ninth tergum ; other lettering as in fig. 1.

Anterior Part. Fig. 3, *a*. A long, stout, rounded, bluntly pointed rod attached anteriorly to the ninth tergo-sternum and with a long bent flange extending freely outwards. *Posterior Part.* Fig. 3, *a*. A long, rather flat plate with a long shelf, and ending in an upwardly directed, bent, pointed process ; there are numerous long and short sensory spines on the outer side, as shown in fig. 3, *c*.

SALIENT DIAGNOSTIC CHARACTERS OF FEMALE TERMINALIA. OVIPOSITOR. SEGMENTS 6, 7, 9 and 10. The segmentation of the abdomen of the ♀ *bovis* is similar to that of the ♂, and the ventral view of the fully extended ovipositor is illustrated in fig. 4, *a*. As the sclerites are all illustrated and are drawn from specimens dissected off, it is unnecessary to describe them. The striking fact about this ovipositor is that it is very long, the intersegmental membrane in particular being much elongated. The main interest in it centres round the distal end, which is illustrated in detail in both dorsal and ventral views in fig. 4, *f*, *h*. It is not possible to be certain of the true identities of the sclerites on this segment, and my interpretation of them may not be the correct one. Tergum 9 consists of two long narrow dorso-lateral plates (fig. 4, *h*). Sternum 9 also consists of two long plates, one on each side of the middle line, their posterior ends diverging, narrowing and then dilating again ; the distal ends are straight, bent over, and form the ventral wall of the genital opening. Tergum 10 appears to be represented by a flask-shaped plate, the distal end rounded and projecting in the form of a ridge ventrally into the wide genital opening, and with the distal ends of sternum 9 forming the egg-guide. Sternum 10 appears to be wanting. The anal cerci (fig. 4, *f*, *h*) consist of a pair of short bluntly rounded processes, displaced laterally, with sensory hairs on their inner surfaces. The genital opening is very wide and occupies most of the space between sternum 9 and tergum 10. The hind intestine is an exceedingly delicate tube ; I have been unable to locate the opening, but I think that it is under tergum 10. The three spermathecae are illustrated in fig. 4, *d*, *e*.

SHORT DESCRIPTION OF THE SEGMENTATION OF THE MALE ABDOMEN AND OF THE MALE AND FEMALE TERMINALIA OF *GASTEROPHILUS* AS EXEMPLIFIED BY THOSE OF *INTESTINALIS* DE GEER

Gasterophilus intestinalis de Geer. SEGMENTATION OF ABDOMEN. SCLERITES. Here again the ventral view of the ♂ abdomen of *intestinalis* showing the segmentation and sclerites is illustrated in fig. 5. The sclerites, etc., at the end of the abdomen are illustrated in fig. 6. Tergum 1 is long and wide and fused with tergum 2. Sternum 1 is a large crescentic plate, and spiracle 1 is located in the membrane close to its antero-lateral edge. Sterna 2, 3, 4 and 5 are very lightly chitinized and are covered with long hairs ; as their structure is shown in the illustration they need not be described. Spiracles 2, 3, 4 and 5 are located in the membrane close to the antero-lateral edges of their respective terga. Tergum

6 (fig. 6) is represented by a narrow plate lying close up against the posterior edge of tergum 7 ; it does not extend round to the sides. Sternum 6 is a very narrow, lightly chitinized, hairy plate which lies over the ninth tergo-sternum (figs. 5 ; 6) ; spiracle 6 is located in the membrane at the antero-lateral edge of tergum 7. Tergum 7 is a wide convex plate which forms the end of the

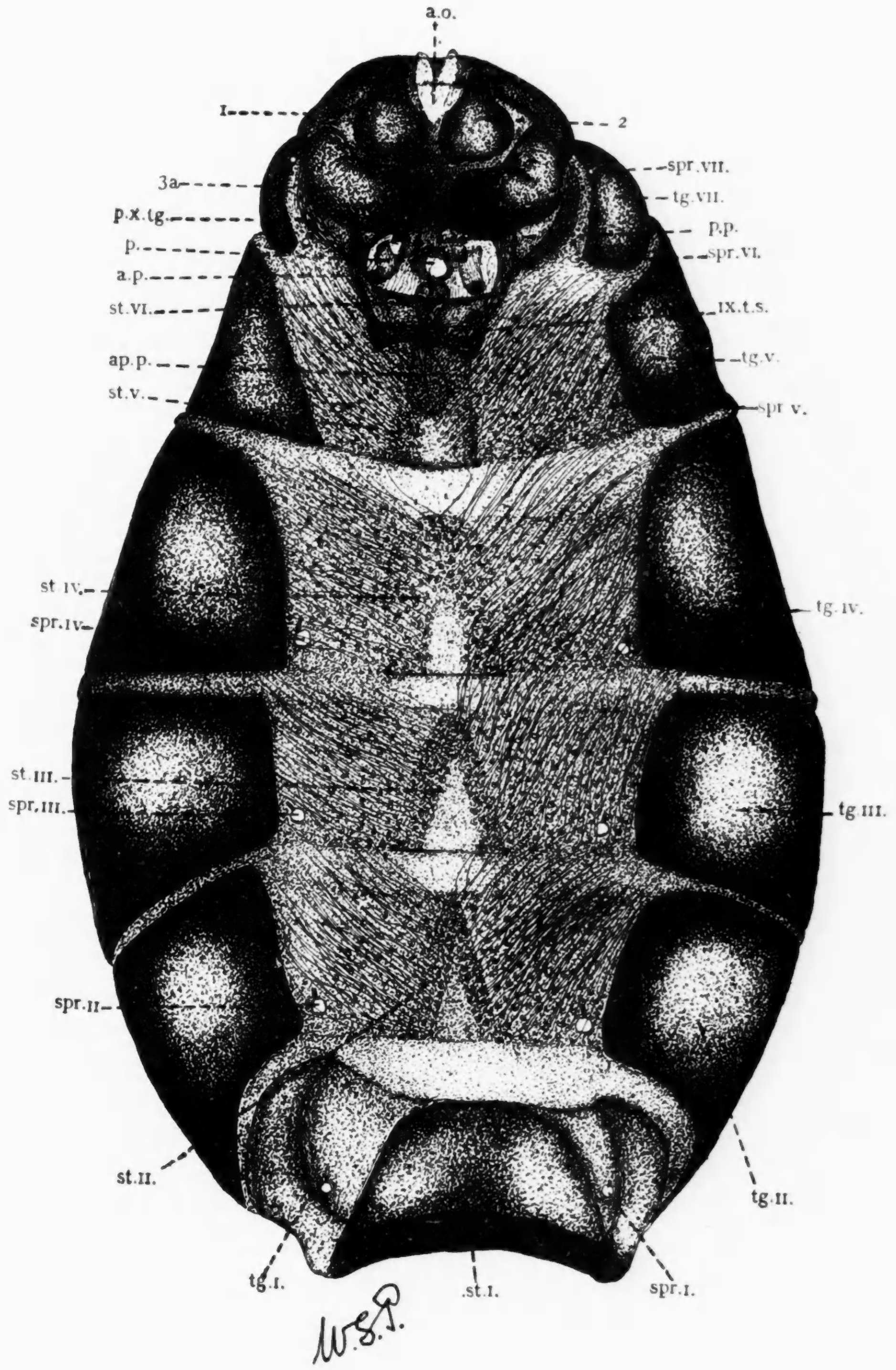

FIG. 5. Ventral view of ♂ abdomen of *G. intestinalis* showing segmentation and sclerites ; 1.—Tenth tergum ; 2.—Anal cercus ; 3*a*.—Distal segment of ninth coxite ; *a.o.*—Anal opening ; *a.p.*—Anterior paramere ; *ap.p.*—Apodeme of phallosome ; other lettering as in fig. 1.

abdomen with tergum 10, to the postero-dorsal margin of which it is fused (fig. 6); antero-ventrally it narrows to a blunt point on each side; spiracle 7 is situated on tergum 7 on its antero-internal edge.

SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. SCLERITES. The ninth tergo-sternum (fig. 7, *b*) is a wide quadrilateral frame-like plate, its dorsal border being the widest part; the lateral and posterior (ventral) arms are narrower, especially the latter, which divide into two processes, the more distal being the longer and narrower; the apodeme of the phallosome is firmly united to the middle of the posterior wall of the wider dorsal part; the proximal

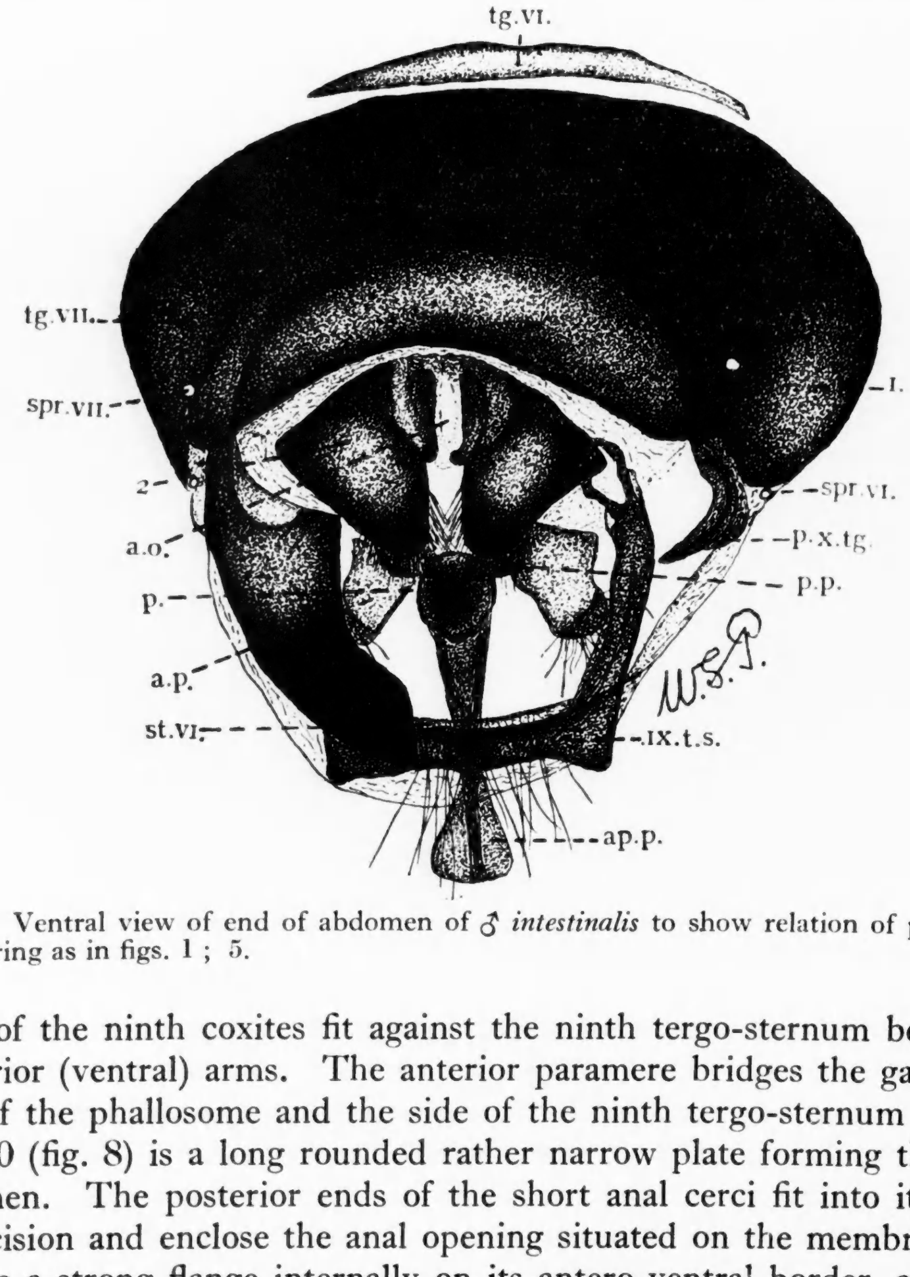

FIG. 6. Ventral view of end of abdomen of ♂ *intestinalis* to show relation of parts to each other; lettering as in figs. 1; 5.

segments of the ninth coxites fit against the ninth tergo-sternum between the two posterior (ventral) arms. The anterior paramere bridges the gap between the base of the phallosome and the side of the ninth tergo-sternum (fig. 7, *b*). Tergum 10 (fig. 8) is a long rounded rather narrow plate forming the apex of the abdomen. The posterior ends of the short anal cerci fit into its postero-ventral incision and enclose the anal opening situated on the membrane. The tergum has a strong flange internally on its antero-ventral border, and a long, bent, pointed process antero-laterally, which abuts against and is fixed by membrane to the side of the ninth tergo-sternum.

APPENDAGES. *Ninth Coxite.* Lateral view. *Distal Segment.* Fig. 8. The distal segment is a broad, heavily chitinized, long, rounded plate, the end bluntly rounded and directed inwards ; it articulates posteriorly against the antero-lateral edge of tergum 10. Ventral view as in figs. 5 ; 6. *Proximal Segment.*

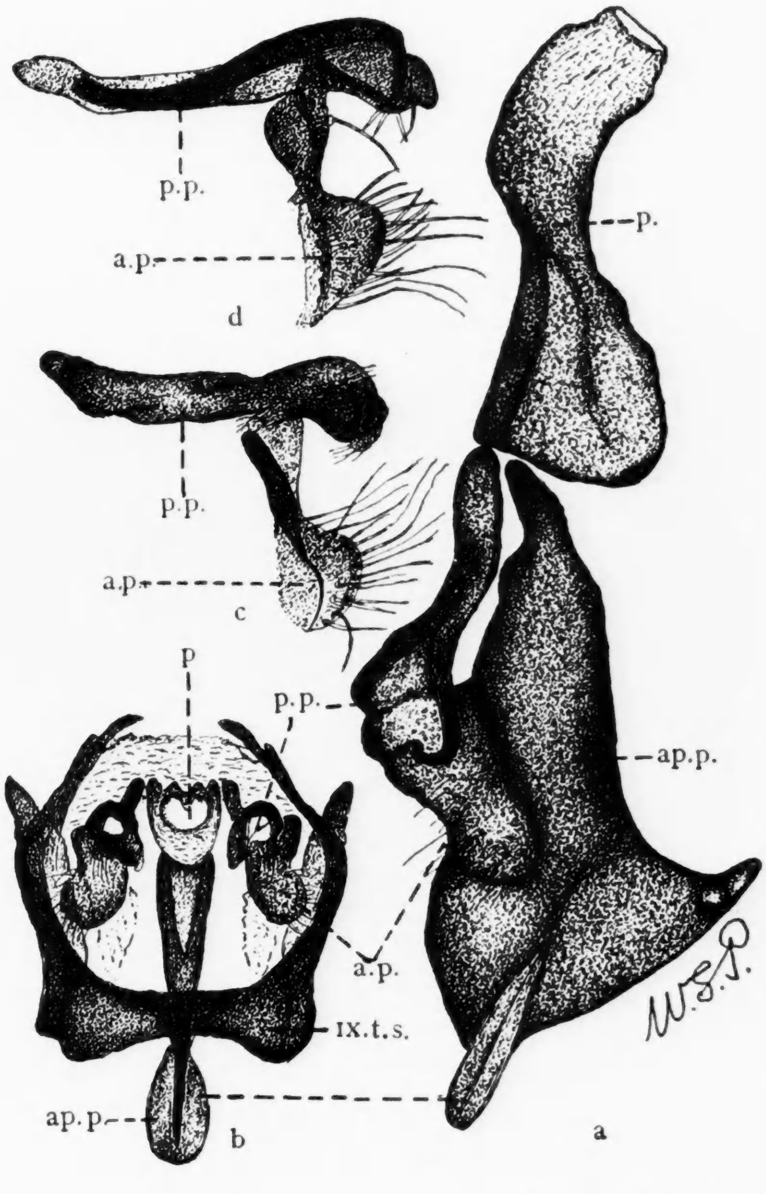

FIG. 7. *a.*—Phallosome, apodeme and one paramere of *intestinalis* in side view ; lettering as in fig. 3 ; *b.*—Ninth tergo-sternum showing normal position (retracted) of phallosome and parameres ; note how the anterior paramere stretches across the side ; *c.*—left paramere in side view ; *d.*—Right paramere in side view ; *a.p.*—Anterior part ; *p.p.*—Posterior part ; other lettering as in fig. 3.

Lateral view. Fig. 8. The proximal segment is a triangular-shaped flat plate ; the apex abuts against the ventro-internal edge of the base of the distal segment and, by a small piece of chitin, with the anal cercus ; the outer angle of the triangle fits against the outer process of the fork of the ninth tergo-sternum, and the inner angle is fused with that of its fellow. *Anal Cercus.* Lateral view.

Fig. 8. A small, rounded, bluntly pointed plate completely separated from its fellow. Ventral view as in figs. 5 ; 6. The two cerci enclose the anal opening which is located on membrane (fig. 6). It is necessary to point out here that some may interpret the plate which I have referred to as tergum 10 as tergum 9, the true tergum 10 being wanting.

Phallosome. Lateral view. Fig. 7, *a.* A stout, simple, rather short, chitinous tube slightly membranous at the end, its walls not very strongly chitinized and without any gadgets. *Posterior Process of Phallosome.* Wanting. *Apodeme of Phallosome.* Lateral view. Fig. 7, *a.* A short, wide, massive plate shaped as shown in fig. 7, *a* ; the antero-dorsal end has a wing-like expansion, which is fused with the middle of the posterior surface of the dorsal part of the ninth tergo-sternum ; the postero-dorsal end is expanded ; the antero-dorsal

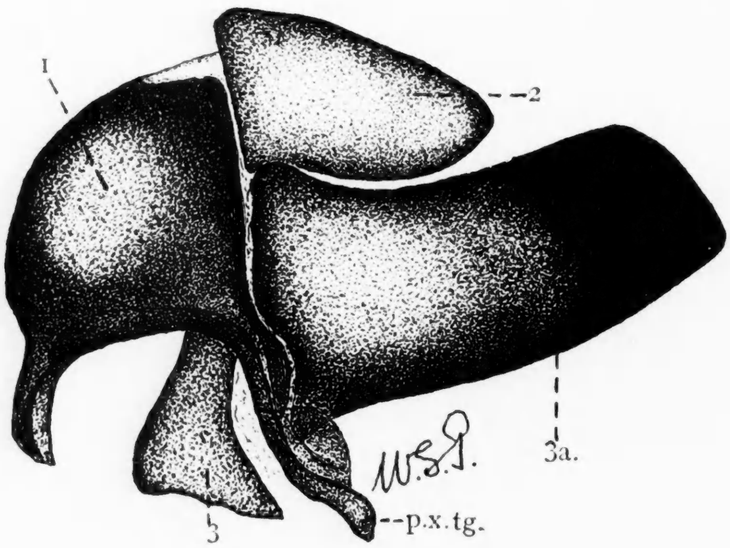

Fig. 8. Lateral view of tenth tergum, anal cercus and ninth coxite of *intestinalis* ; lettering as in figs. 1 ; 5.

view of the apodeme is shown in figs. 5 ; 6 ; 7. *Sperm Pump Sclerite.* Wanting. *Parameres.* Lateral view. *Anterior Part.* Fig. 7, *a, c, d.* The distal end of the anterior part is a rounded hollow plate armed with numerous long hairs ; it is united to the posterior part by membrane as shown in fig. 7, *c, d. Posterior Part.* Fig. 7, *a, c, d.* A long plate dilated distally and bearing many sensory hairs and united by membrane to the base of the phallosome. As noted above, the anterior part bridges the gap between the phallosome and the dorsal wall of the ninth tergo-sternum.

Salient Diagnostic Characters of Female Terminalia. Ovipositor. Segments 6, 7, 9 and 10. The segmentation of the ♀ *intestinalis* is similar to that of the ♂. The ventral view of the fully extended ovipositor is illustrated in fig. 9. The ovipositor of *intestinalis* in contrast to that of *Hypoderma bovis* is short and wide, and is always seen extended (except for segments 9 and 10)

under the abdomen, almost parallel to the body. The various sclerites are illustrated in fig. 9, and here again it is possible that my interpretation of them may not be correct. Tergum 6 is a large plate covering the whole of the dorsal surface, while sternum 6 is a Y-shaped plate ; note the positions of spiracles

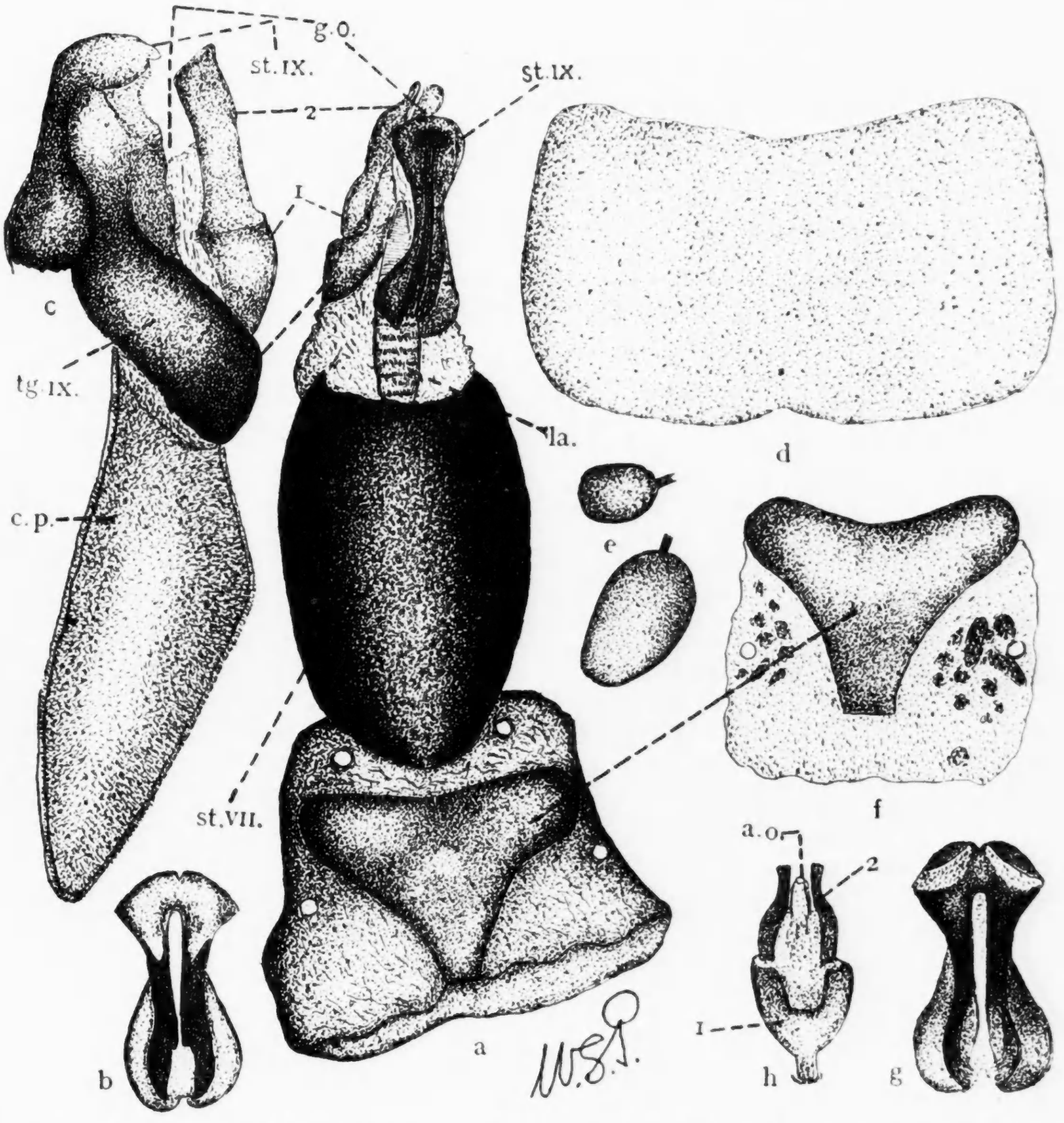

FIG. 9. *a.*—Ventral view of fully extended ovipositor of *intestinalis* showing sclerites ; note egg containing larva (*la.*) at end ready to be attached to a hair ; lettering as in fig. 4 ; *b.*—Ventral view of sternum 9 ; *c.*—Ninth tergum and sternum, tenth tergum and anal cerci in lateral view ; note the large reticulated chitinous plate (*c.p.*) attached to the ninth tergum ; *d.*—Sixth tergum ; *e.*—spermathecae ; *f.*—Sixth sternum ; *g.*—Dorsal view of ninth sternum ; *h.*—Dorsal view of tenth tergum and anal cerci.

6 and 7 in fig. 9, *a*. Tergum 7 and sternum 7 are fused, forming a dense strong tube ; the two edges of the tergum are slightly separated. Tergum 9 (fig. 9, *c*) is a short wide plate extending laterally, while sternum 9 is a highly modified plate dilated at its end, with two strong backwardly bent bars running its whole length ; there are a number of sensory hairs at the end on the dorsal side ; the plate forms an excellent egg-guide. Tergum 10 (fig. 9, *c*, *h*) is a cup-shaped

plate, and articulating with its distal end are the short rather narrow anal cerci. There are only two spermathecae, one larger than the other. The interest attached to this ovipositor is the strong long tube-like seventh tergum and sternum, which is attached to the sixth segment by a very short intersegmental membrane, and is clearly modified to strengthen and maintain the ovipositor in the middle line during the act of oviposition. The genital opening is also wide, but the anal opening is small and lies between the anal cerci.

THE SYSTEMATIC POSITION OF *Hypoderma* AND *Gasterophilus*. Before referring to the systematic position and relationships of these two genera, it is necessary to note briefly the structure of the ovipositor of each in relation to the method of oviposition, and its bearing on the structure of the ♂ terminalia. As is well known, both *H. bovis* and *G. intestinalis* deposit one egg at a time, attaching it to the hair of the host ; the former fixes the grooved pedicel close to the base of a hair out of sight, whereas the latter attaches it by a groove (without pedicel) to the middle or end of a hair in full view. Hadwen (1912) has observed the method of egg-laying in the case of *bovis* and describes it ' as a frenzied sort of process, the fly striking twenty or thirty times rapidly (no accurate account kept) then leaving the animal for fifteen minutes or so, when the process would be repeated.' He noted that the ' egg comes out of the ovipositor with the grooved end first ; so that the ovipositor must in some way open out the groove of the egg, adjust it to a hair, surrounding it with the gummy substance before it fully comes out.' The study of the ovipositor shows that it is well adapted to this particular method of oviposition. It can rapidly be extended to its full length (or less), depending on the length of the hair of the host, and is evidently only extruded completely when the fly holds the hairs momentarily with its long legs. Owing to its great flexibility it can be wormed down between the hairs to the base. The structure of the ventral (internal) surface of tergum 10 clearly suggests, I think, that the groove is opened by the ridge on the tergum, as the egg is pressed against it by the end of sternum 9, the two forming a perfect egg-guide.

The ♀ *G. intestinalis,* which I have repeatedly watched ovipositing and pairing, flies slowly and noiselessly round a horse, and, every now and then approaching the skin, momentarily grasps the hairs with its long legs. The end of the rigid, stout ovipositor, which is always carried extended and parallel to the ventral surface of the body, with an egg close to the genital opening ready to be laid, is applied to a hair, and an egg is extruded, with the grooved side towards the hair which fits into it.

The ♂ terminalia of each are specially adapted to function with their respective ovipositor. The distal segments of the ninth coxites of *H. bovis* are very small and adapted to grasp the rather slender, lightly chitinized end of the ovipositor, and the most which the small fused anal cerci could do would be to press the end against the coxites. The phallosome is long, the ejaculatory duct opening at the end of a long membranous part which can be made rigid.

The ♂ terminalia of *G. intestinalis*, on the other hand, are strikingly different, and are structurally adapted to function with a shorter, stouter and much more rigid ovipositor. The distal segments of the ninth coxites are here powerful pincers, which grip the tube-like seventh segment as in a vice ; the two separate anal cerci press the end against the coxites, and the short simple phallosome is erected between the coxites and readily passes into the genital opening.

Although both species exhibit in their ♂ terminalia two characters in common, viz., distal segments of the ninth coxites which grasp laterally, and small anal cerci which play only a minor part in grasping, in all other respects they are quite distinct and show no structural relationships. Each genus, therefore, stands by itself, and each is best retained in a separate subfamily, the Hypodermatinae and Gasterophilinae respectively. Both are very ancient types, exhibiting the ancestral form of ninth coxite and anal cerci, and of the two *Gasterophilus* appears to be the older as judged by the structure of the terminalia.

Some authors place *Hypoderma* in the family Tachinidae, while others place the genus in the subfamily Oestrinae (Oestridae), allying it with *Oestrus* L. and *Cephalemyia* Lat. It is impossible as yet to express an opinion as to the relationship of *Hypoderma* with the Tachinidae. With regard to the relationship of *Hypoderma* to *Oestrus*, *Cephalemyia*, etc., I shall refer to it again when describing the terminalia of these genera.

With regard to *Gasterophilus*, it will be remembered that Girschiner (1896) placed the genus in his family Anthomyidae (Muscidae-Anthomyidae auctores), stating that it stands completely isolated in it and is allied as a very old form to the Acalypteratae. In other words, he would place *Gasterophilus* between the Anthomyidae and the Acalypteratae, whatever that may mean. At present I can see no relationship between that section of the Anthomyidae (Coenosinae, etc.)—as understood by Girschiner—and *Gasterophilus* ; but I cannot express a final opinion until I have studied the terminalia of more of the species of Anthomyidae. I am not yet able to express any opinion as to the relationships of *Gasterophilus* with the Acalypteratae. In the next part of this series I shall describe and illustrate the terminalia of Cuterebrinae, as exemplified by those of some species of *Cuterebra* and *Dermatobia hominis*.

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(*To be continued*)

ON THE MALE TERMINALIA OF SIMULIIDAE

BY

E. G. GIBBINS

(*Received for publication* 17 *May*, 1935)

INTRODUCTION

The significance of the male terminalia in the classification of *Simulium* has long been realized ; but the uncertainty of the precise homologies of its complicated structures has led to much confusion in nomenclature, and the worker is apt to become bewildered by the diverse terminology now in use.

Dr. C. Lundström (1911) first studied the male terminalia of *Simulium* when describing species from Finland, and gave several figures of this organ. His drawings, however, were not in sufficient detail, and it was Dr. F. W. Edwards (1915) who established the great importance of the male terminalia during a study of British Simuliidae. Later (1931) he extended his studies to the *Simulium* of Patagonia and South Chile. Following closely on Dr. Edwards's early paper, Mr. A. W. J. Pomeroy, M.B.E., (1916) published an account of the North American Simuliidae, figuring their terminalia. Some years later, Dr. I. M. Puri (1932) described the various parts of the male terminalia when embarking on an extensive study of the Indian Simuliidae. Nevertheless, there still remains considerable doubt as to the true homologies of the various structures ; and it is hoped that the present contribution will throw further light on this complicated subject. The study is intended to serve as a basis for further work, and was undertaken mainly in order that a definite and more accurate terminology may be adhered to in the future.

The study is based on the examination of a large collection of *Simulium* from Uganda. Some of the species are extremely small and are no more than the size of a midge, while others, of which *Simulium nili* Gibbins is an example, are almost as large as a small house-fly. Many of the species are so closely related that they can only be distinguished from each other by a detailed examination of the terminalia. The drawings have been made with the aid of the camera lucida from permanent preparations mounted in canada balsam *without compression.* In carrying out the intricate dissections, I have followed the procedure of Professor W. S. Patton, whose delicate technique I recently had the pleasure of learning, and where practicable I have also adopted his terminology (Patton, 1932).

SEGMENTATION OF THE MALE ABDOMEN OF *SIMULIUM NILI*

The abdomen (fig. 1) consists of the usual nine visible segments ; the tenth is only seen in cleared preparations. The first abdominal segment is similar in both the male and the female, and bears a conspicuous narrow collar which

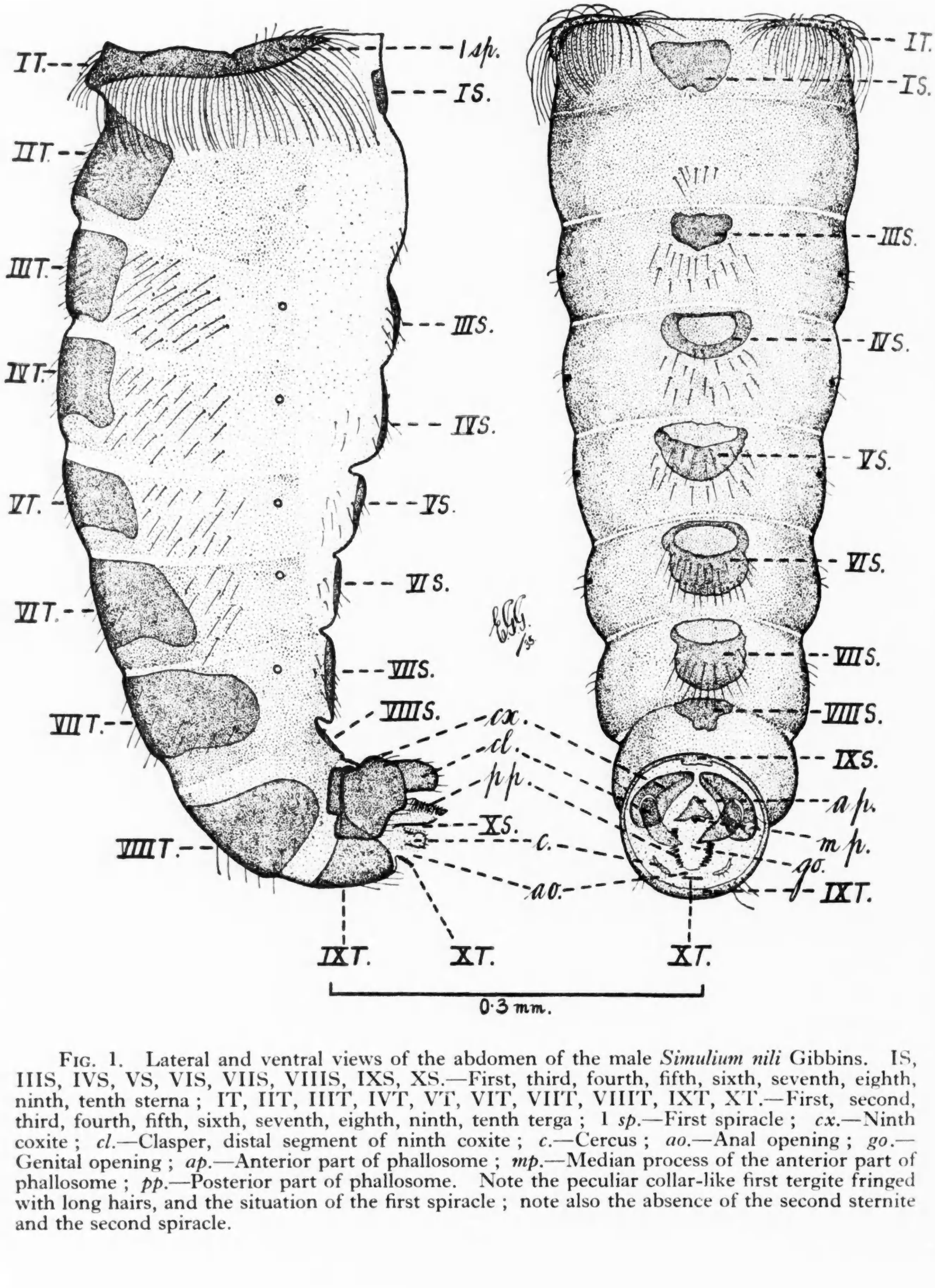

FIG. 1. Lateral and ventral views of the abdomen of the male *Simulium nili* Gibbins. IS, IIIS, IVS, VS, VIS, VIIS, VIIIS, IXS, XS.—First, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth sterna ; IT, IIT, IIIT, IVT, VT, VIT, VIIT, VIIIT, IXT, XT.—First, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth terga ; 1 *sp.*—First spiracle ; *cx.*—Ninth coxite ; *cl.*—Clasper, distal segment of ninth coxite ; *c.*—Cercus ; *ao.*—Anal opening ; *go.*—Genital opening ; *ap.*—Anterior part of phallosome ; *mp.*—Median process of the anterior part of phallosome ; *pp.*—Posterior part of phallosome. Note the peculiar collar-like first tergite fringed with long hairs, and the situation of the first spiracle ; note also the absence of the second sternite and the second spiracle.

encircles the dorsal and lateral margin of the abdomen. This structure is peculiar to *Simulium* and was thought to belong to the thorax. It is strongly chitinized and is composed of three sections (fig. 2, *a*). The central part, which is about three times as long as the lateral, surrounds the dorsal surface and appears as a broad band, while the lateral sections are convex outwardly; the whole is clothed with long outstanding hairs, giving it the appearance of a fringe. Edwards (1915) stated that he was convinced that it represented the first abdominal tergite, but gives no evidence (apart from the fact that he made careful dissections of cleared specimens) in support of this hypothesis. Nevertheless, his assumption appears to be correct, for by following the trachea

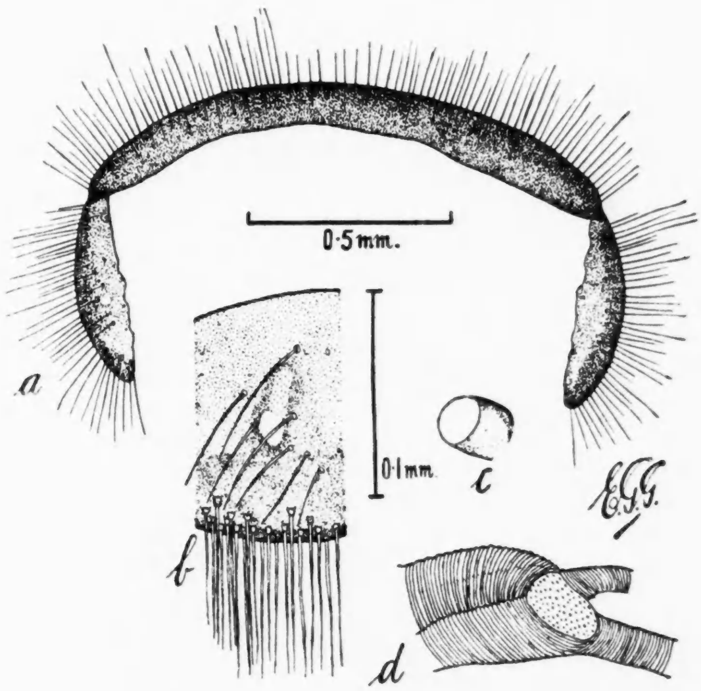

FIG. 2. *Simulium nili*. *a*.—First tergite; *b*.—Part of lateral section of first tergite enlarged to show the first spiracle; *c*.—Open spiracle of third segment; *d*.—Converging trachea and closed spiracle of second segment. (*b*, *c* and *d* are of the same magnification.)

the writer has been able to demonstrate the presence of a small open spiracle (fig. 2, *a*, *b*) in the median area of the upper surface of the lateral section, thereby associating it with the first abdominal segment. Taylor (1902) studied the tracheal system of *Simulium* but overlooked the first spiracle. He found open spiracles in the third to seventh segments inclusive, and was surprised by their absence from the first and second, particularly as he had seen tracheal remnants of these segments withdrawn and left attached to the cast skin of the pupa when the fly had emerged. The first abdominal sternite is normal and comparatively large and irregular in shape.

The second segment is also unusual. There is a well-marked tergite but no sternite, its place being occupied by about six small spines. Furthermore, there is no open spiracle, though the trachea shows an opening below the cuticle

in the normal position of the spiracle. The converging trachea and the spiracle are depicted in fig. 2, *d*.

Segments 3–7 inclusive show strongly chitinized open spiracles (fig. 2, *c*) and bear well-marked sternites in the form of small plaques. Spiracles are absent

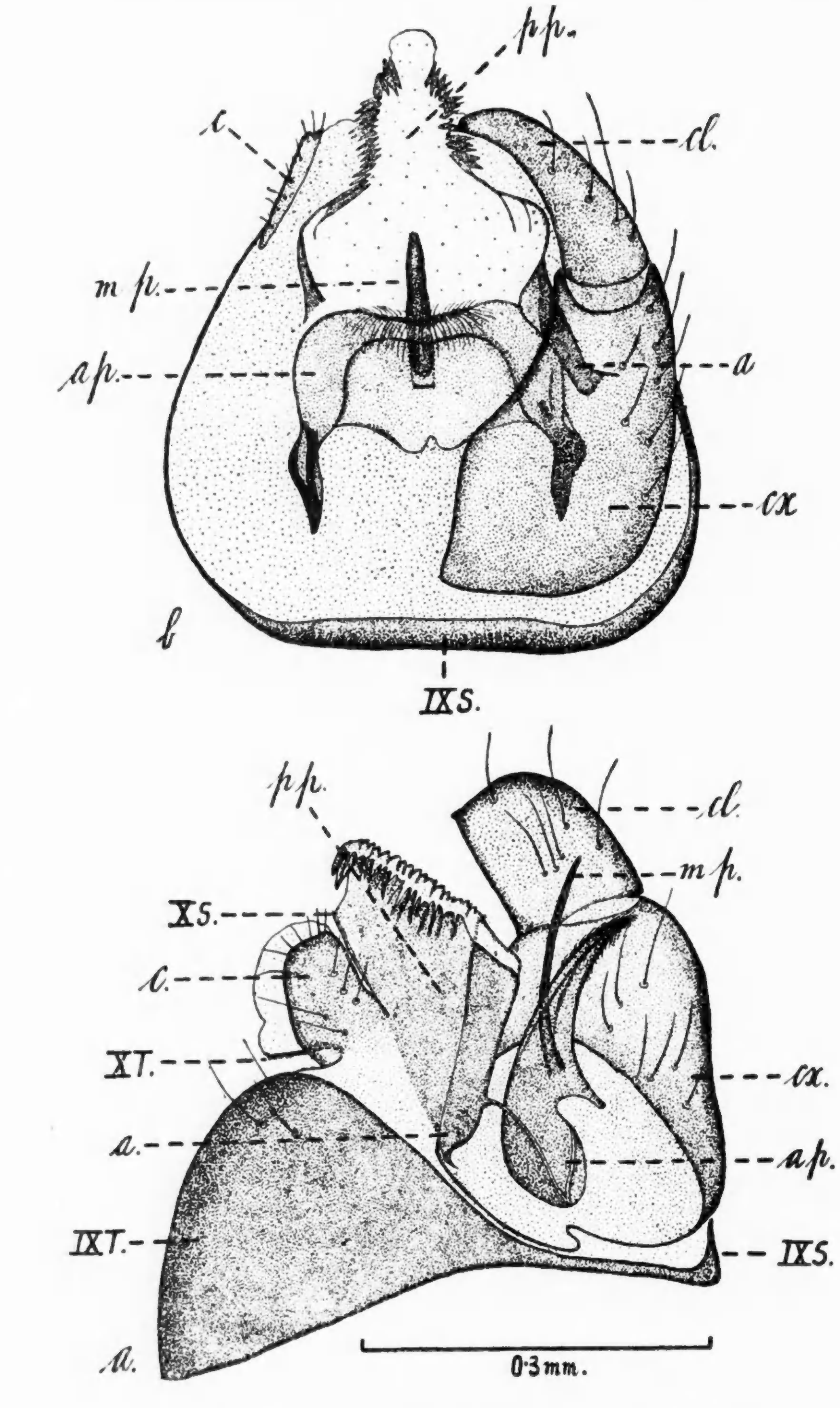

FIG 3. *a.*—Lateral view of male terminalia (ninth segment) of *S. nili* ; the near coxite and the clasper have been dissected away and the apodeme (*a*) broken off from the coxite at its base. *b.*—Ventral view of same ; note the anterior part of the phallosome with its distal end directed forwards, giving it an entirely different appearance ; note also the large pocket of the posterior part within which lies the genital opening. Lettering as in fig. 1. (See fig. 5 for enlarged drawing of the phallosome.)

from the remaining segments. The eighth sternite is similar in size to the third, and all the tergites are large and take the form of a wide conspicuous band.

TERMINALIA

NINTH SEGMENT (fig. 3, *a*, *b*)

The ninth segment is large and triangular in lateral aspect. It almost entirely envelopes the terminal appendages, holding them in a vertical position. In *S. nili* the ninth sternite is usually well-marked, but in other species this character is not so obvious and the sternite is reduced to little more than a narrow band. The ninth segment carries the coxites with their distal appendages, the phallosome, the tenth tergite and sternite together with the small anal cerci. The coxites take up a ventro-lateral position with the phallosome, which is attached to their base on either side, situated in between ; they protect and usually completely obscure the anterior part of the phallosome from lateral view. Immediately behind, and in contact with the posterior part of the phallosome, lies the tenth sternite (fig. 4) ; this is a delicate membrane, spiculate

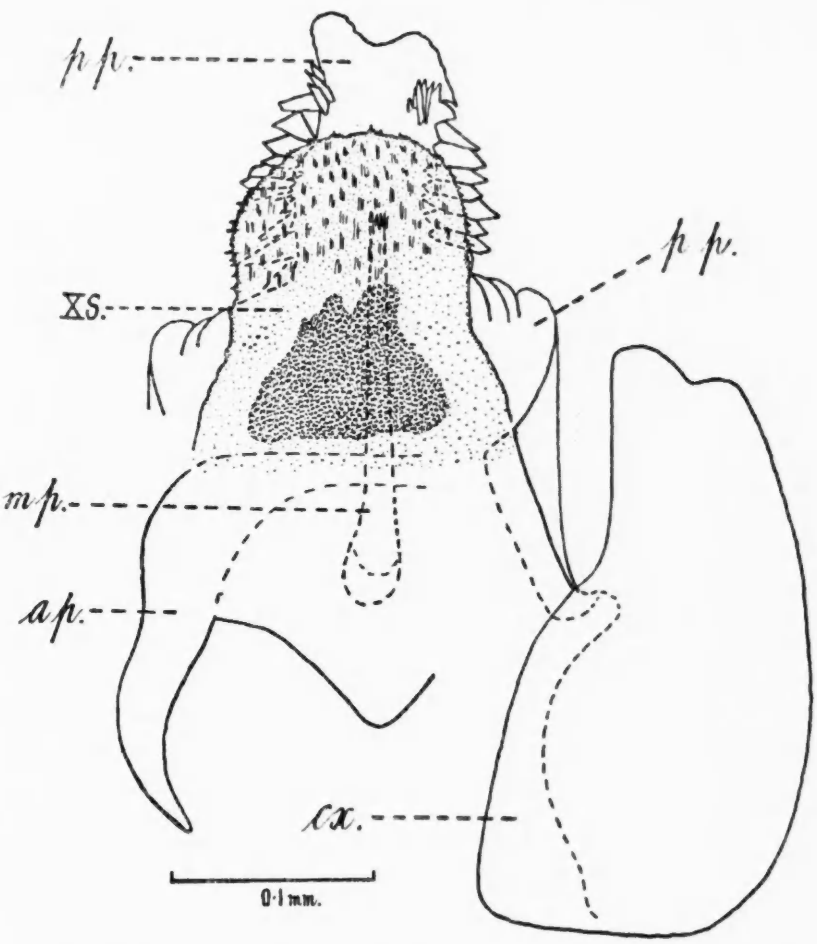

FIG. 4. Dorsal (posterior) view of male terminalia of *S. nili* drawn to show the position and structure of the tenth sternite.

distally with a strongly chitinized triangular patch. Next is the anal opening bounded on either side by conspicuous cerci ; and finally the tenth tergite, which is small and strongly chitinized, taking up a dorsal (posterior) position.

PHALLOSOME

A careful examination of the organ which lies hidden from lateral view between the coxites and which hitherto has been something of a mystery—having been referred to as the adminiculum by Pomeroy (1916), the aedoeagus by Tonnoir (1925), the ventral plate (? ninth sternite) by Edwards (1931), and the intercoxal-piece by Puri (1932) and the writer (1934)—has elicited the fact that it is no other than the anterior part of the phallosome.

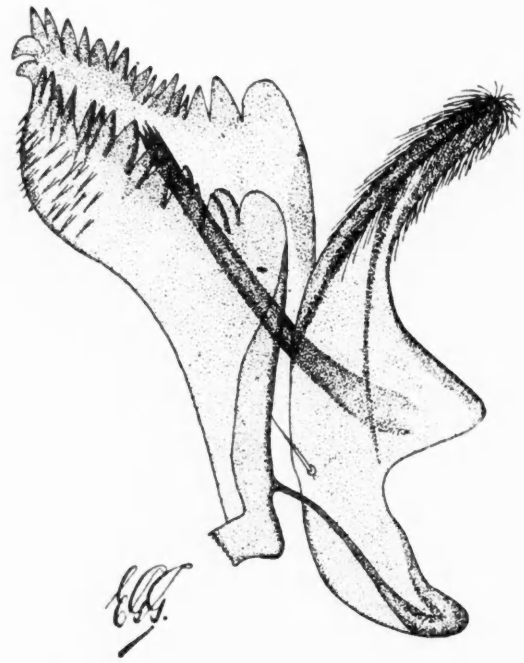

FIG. 5. Lateral view of phallosome of *S. nili* (magnification as in fig. 6, *c*).

It will be seen in fig. 5 that the phallosome is divided into an anterior and a posterior part ; these two sections are joined together by a delicate strip of chitin which is often broken in the process of dissection. Of the two, the anterior part is the most pigmented ; the posterior part in many cases is principally composed of semi-transparent membrane. The anterior part is provided with hairs distally ; these are sometimes supplemented by short stout spines (*S. damnosum*, fig. 6, *a*) and in some cases by short teeth (*S. hargreavesi*, fig. 6, *c*). Edwards (1931) noted the presence of a strong median keel, and Tonnoir (1925) speaks of this part as resembling the bridge of a violin. Its most striking features are the strongly chitinized, downward-projecting basal processes situated on either side, which are clearly seen when the organ is viewed

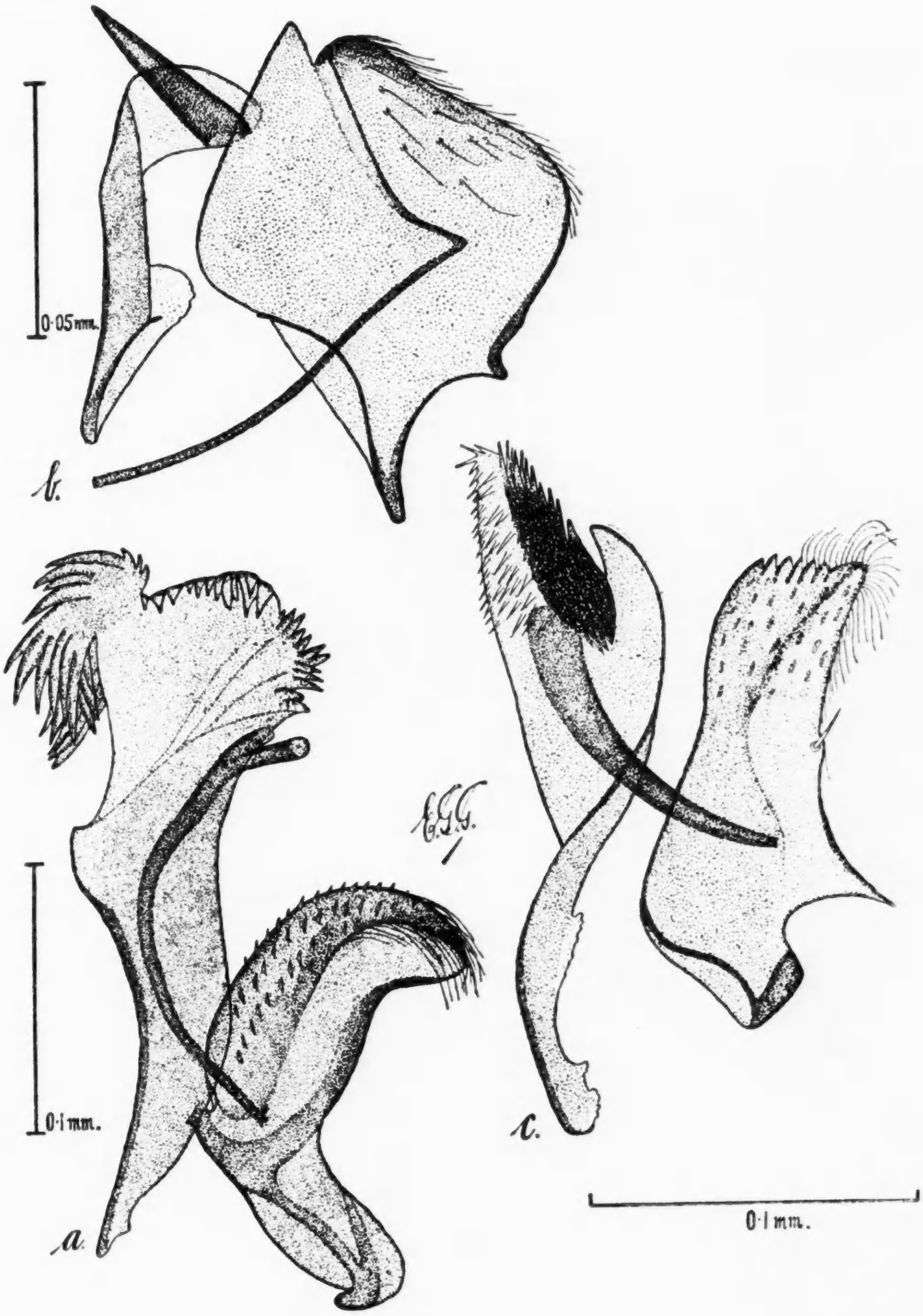

FIG. 6. Lateral view of phallosome of three species which, with *S. nili* (fig. 5), illustrate its structure in four different groups of *Simulium* (far side of posterior part not shown). *a.*—*S. damnosum* Theo.; *b.*—*S. hirsutum* Pom.; *c.*—*S. hargreavesi* Gibbins. Note the large anterior part and small posterior part in the case of *S. hirsutum*; the distal teeth of the posterior part are absent and replaced by a pair of chitinized cones.

in a ventral aspect (fig. 7). From these processes arise the chitinous membrane which forms the only attachment to the posterior part. When examined from the side, its widely different forms are more readily appreciated (figs. 5 ; 6).

Attached to the dorsal membrane of the anterior part of the phallosome in its median sub-basal area is a structure which varies considerably in shape

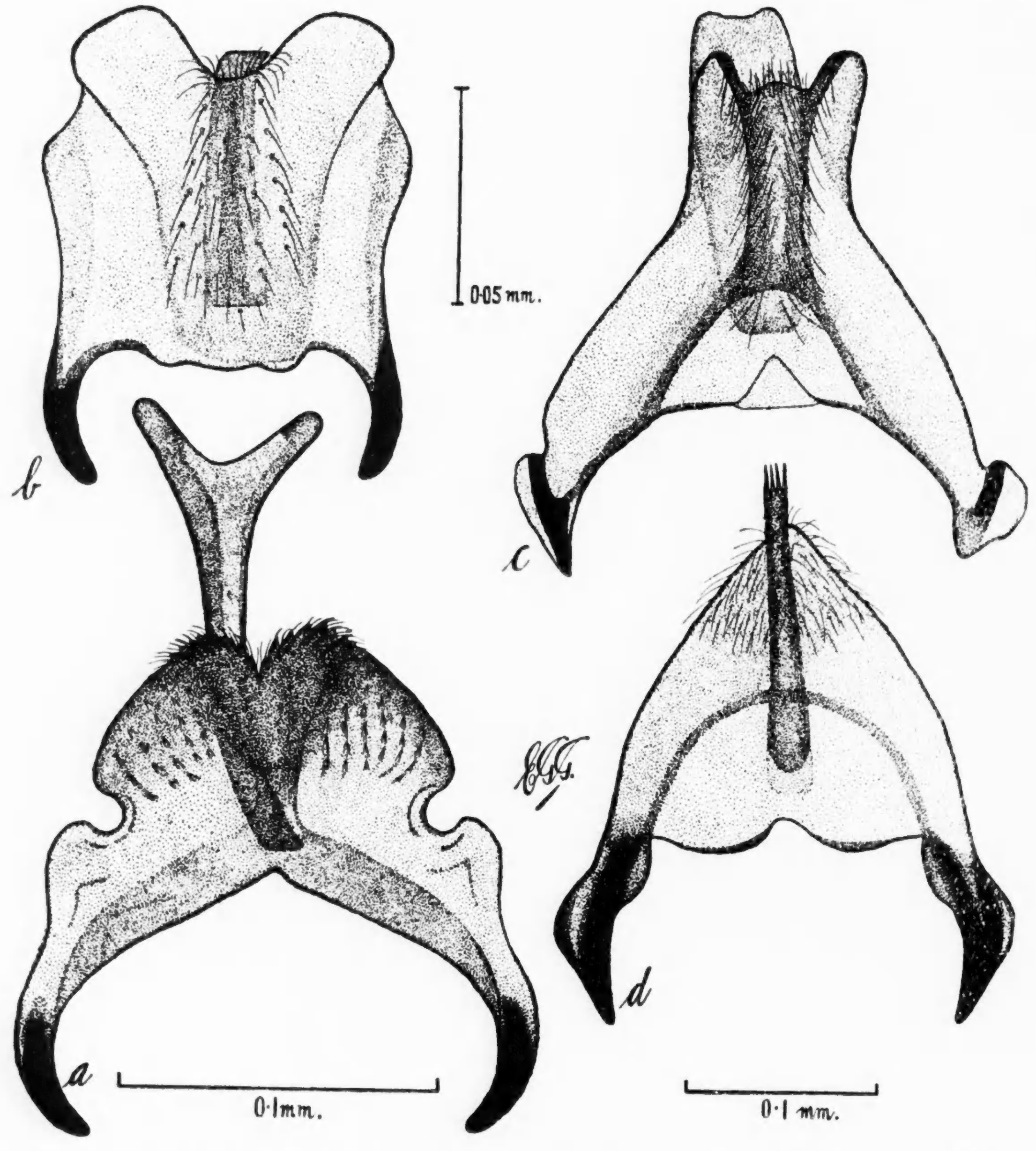

FIG. 7. Ventral view of anterior part of phallosome. *a.—S. damnosum* ; *b.—S. hirsutum* ; *c.—S. hargreavesi* ; *d.—S. nili*. (*a* and *c* are of the same magnification.)

but is apparently a constant group character. It may be the penis, but as its true homology is unknown I prefer to refer to it tentatively as the median process. Figs. 5, 6 and 7 show lateral and ventral views of the different forms. In *S. hargreavesi* it is short and broad, while in *S. nili* it is narrow and cylindrical,

tapering to a brush-like point. In *S. damnosum** it is long and peculiarly forked, while in *S. hirsutum* it is broad and particularly long. Its normal position at rest appears to be with its distal end directed into the large membraneous pocket of the anterior part (figs. 5 ; 6).

The apodeme forms the support of the posterior part of the phallosome and also provides the only means of support of the anterior part (figs. 3 ; 5). It is connected to the coxite at its base.

The study has shown the existence of two different types of the posterior part of the phallosome. One type consists of a large membraneous pocket armed with strong teeth or spines along its distal edge, while in the other type, represented by *S. hirsutum* in fig. 6, *b*, the membrane is very much reduced and the teeth are absent and replaced by a pair of large chitinized cones, which the writer has termed 'parameres' in previous studies.

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*In a previous paper (1933) this structure was wrongly referred to as the intercoxal-piece.

THE PRESERVATION IN THE TROPICS OF BLOOD SMEARS STAINED BY THE ROMANOWSKY STAIN

BY

J. L. PAWAN, M.B.E., M.B., Ch.B., D.P.H.

(*Government Bacteriologist, Trinidad, British West Indies*)

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In a previous communication (1928) it was pointed out that any of the modified Romanowsky stains (Leishman, Wright, Giemsa, etc.), if kept in solution in a Pyrex bottle, suffers no deterioration and retains its staining properties for years under tropical conditions. The writer has been using these stains, made in solution locally and kept in Pyrex bottles, for over four years, and every tinctorial detail of blood cells and parasites has been clearly differentiated. The preservation of these preparations was, however, always unsuccessful. Fading of blood smears after staining by any one of these stains is a well-known and distressing feature of laboratory work in the tropics. While in temperate climates such preparations may retain their original appearances for 10, 15 or even 20 years, in the tropics fading begins in periods varying from 3 to 6 months, and in about 9 months—in Trinidad, at all events—the nuclei of cells and parasites appear indistinguishable and colourless. Every method recommended was resorted to, but without success—e.g., mounting in neutral immersion oil and in various so-called neutral media, ringing with various ' neutral ' substances, storage under calcium chloride in the dark and at reduced temperatures, etc. But fading invariably followed, until the following simple device was employed. A Coplin's jar or other suitable container is filled with xylol (B.D.H.). Solid paraffin (M.P. ca. 60°) is made into fine shavings and added to the xylol at room temperature with continual stirring until no more can be dissolved. The mixture of xylol and paraffin is then placed in the incubator at 37·5° C. for an hour or two to complete further solution, after which fresh shavings are added up to saturation. When it is desired to preserve a preparation, proceed as follows : Wash off with xylol the oil left on the stained, unmounted slide ; remove the saturated mixture of xylol and paraffin from the incubator and uncover to hasten cooling ; allow the dissolved paraffin *to begin* to solidify, as indicated by an opacity in the previously clear xylol; then dip the stained slide into the mixture and remove rapidly so as to envelop with a uniform coat of dissolved paraffin the entire slide with the stained smear in the middle. Allow the xylol to evaporate, and a coat of dry, solid paraffin will cover the whole slide. With this coat the preparation can be stored for further examination. When this is required, all that is necessary is to dip the slide with its paraffin coat into another jar of xylol (without paraffin) so as to remove all trace of the paraffin, allow the xylol to evaporate, and examine with the oil immersion in the usual way. This process

of paraffin coating and examination can be repeated several times. In this way the writer has preserved blood smears with all forms of malarial parasites, smears from cases of leukaemia and pernicious anaemia, etc., for as long as 5 years and 3 months, and the original tinctorial appearances of the blood cells and parasites have remained unaffected, even though the slides were left exposed to the sunlight on the laboratory bench.

SUMMARY

1. Under tropical conditions in Trinidad, storage of the modified Romanowsky stains in Pyrex glassware will preserve the original staining properties for years.

2. Protection of these stained smears by a coating of solid paraffin, previously dissolved in xylol, will preserve the original tinctorial appearances of blood cells and parasites for over 5 years in Trinidad.

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MALARIA STUDIES IN GREECE

THE MALARIA INFECTION RATE IN NATURE AND IN THE LABORATORY OF CERTAIN SPECIES OF *ANOPHELES* OF EAST MACEDONIA*

BY

M. A. BARBER

AND

J. B. RICE

(*Received for publication* 11 *June*, 1935)

The chief purpose of the work here reported was to determine the infection rate of *Anopheles* in nature, by species, locality, seasons and years. In addition, a study of the susceptibility of different species to malaria parasites was made by means of laboratory experiments. For these several purposes many dissections were necessary in order to afford significant numbers, and many thousands were performed. In order to distribute the personal error in a work essentially comparative, nearly all the examinations of dissected mosquitoes and the verification of all positives were done by one person (M.A.B.).

The region in which our mosquito collections were made is largely that included between the lower part of the Nestos River and the valley of the Struma. This region contains two large plains : the Plain of Chrysoupolis, which borders the Nestos River and the Aegean Sea ; and the Drama-Philippi Plain, which is situated well in the interior, its seaward border being at least 4 km. from the sea and separated from it by a range of mountains. In these plains and in the mountains immediately adjacent to them are situated the greater number of the villages investigated. Nearly all these villages lie within 50 km. of the city of Cavalla, in which our laboratory is situated. With regard to the natural infection rate of *Anopheles*, the region of our study is probably representative of all northern Greece, except perhaps some localities less abundantly supplied with domestic animals.

Our work was continued through the period April, 1932, to December, 1934, and included three full summers and autumns, one full winter (1932-33), and some additional months in the springs and winters of different years (see Table II).

The species of *Anopheles* studied, *A. elutus* (*sacharovi*), *A. maculipennis* (varieties *messeae* and *typicus*), *A. superpictus*, *A. hyrcanus*, *A. algeriensis* and *A. bifurcatus*, are found throughout the entire region, although naturally in varying proportions. Malaria is endemic in this part of Greece, and all three of

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*The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

the commoner species of malaria parasite, *P. vivax*, *P. falciparum* and *P. malariae*, are abundant.

I. INFECTION IN NATURE

Table I presents the results of the dissections, stomach and salivary glands, of mosquitoes bred in 1932, including those surviving in a hibernating or semi-hibernating condition into 1933. Dissections of April, 1932, of mosquitoes surviving the preceding winter, are shown in Table II. All localities are included in Table I, except one (Kerkini-Lithotopos) in which the survey, begun late in the season, was made essentially a winter study. The dissections of this locality will be described separately in a paper on the winter transmission of malaria*.

Under the caption ' No. dissected ' (Table I) are included all mosquito dissections, whether of stomach, of salivary glands, or of both. ' Percentage positive ' includes all positives, whether for oöcysts or sporozoites ; a mosquito with both oöcysts and sporozoites counts as one positive. Of total dissections, salivary glands were dissected in about 99 per cent., and stomachs in about 80 per cent. Throughout this paper, oöcyst rates are based on the number of stomachs examined, and sporozoite rates on the number of gland dissections. The sporozoite rate of 1932 is shown separately in Table II.

TABLE I

Infection rate in nature, by species and months, of *Anopheles* bred in 1932, including those hibernating in 1933

Month	*A. elutus*		*A. maculipennis*		*A. superpictus*		All species†	
	No. dissected	Percentage positive	No. dissected	Percentage positive	No. dissected	Percentage positive	No. dissected	Percentage positive
1932								
May ...	973	0·8	66	0·0	2	0·0	1,043	0·8
June ...	1,723	1·1	291	0·3	6	0·0	2,020	1·0
July ...	1,726	2·2	387	0·5	121	0·8	2,236	1·9
August	1,592	2·4	525	0·6	583	4·5	2,703	2·6
Sept. ...	966	6·0	436	0·2	956	4·0	2,371	4·2
Oct. ...	505	3·2	841	0·4	1,209	1·5	2,727	1·3
Nov. ...	721	1·9	753	0·1	1,306	1·5	2,892	1·2
Dec. ...	391	1·8	506	0·0	1,207	0·6	2,319	0·6
1933								
Jan. ...	189	4·8	130	0·0	738	0·7	1,067	1·3
Feb. ...	178	1·7	144	0·0	241	0·4	563	0·7
Total all months	8,964	2·37	4,079	0·27	6,369	1·82	19,941	1·71

†Among ' all species ' are included : *A. hyrcanus*, 11 ; *A. algeriensis*, 253 ; *A. bifurcatus*, 255. All these were negative (see Table II).

*Our work has afforded some interesting by-products, the detailed description of which would make this paper too cumbersome. These subjects will be described in separate papers and will be omitted or only briefly summarized here.

Comment on Table I. In the totals, the index of *elutus*, 2·37, is highest; that of *superpictus*, 1·82, is nearly as high. The seasonal incidence of positives, however, is quite different. *Elutus* reaches 0·8 per cent. in May, while *superpictus* does not reach a similar height until July. The numbers of *superpictus* dissected in the earlier months are few, because this species is comparatively rare in the spring. Both species reach their maximum of infection in midsummer.

The three species shown in the Table begin early to accumulate fat for winter, and the average longevity in autumn is doubtless greater than in the summer. Their parasites remain in them, so that autumn positive rates are not strictly comparable with those of summer. The danger of malaria transmission, also, may not run parallel with the parasite rate of the insects, for biting activity is lessened in autumn. A second factor may enter—a decreased infectivity of sporozoites. This factor, less easy to evaluate, will be discussed in another paper. These considerations apply in greater degree to the winter parasite rates of anophelines. The high January rate of *elutus* is partly due to the chance occurrence of 6 positives from one village among the small numbers examined.

The parasite rate of *maculipennis* is very low, and the positives were scattered from June to November. Apparently no variety of *maculipennis* is found in his region except *messeae* and *typicus*. Among hundreds of ovipositions in he laboratory and many surveys of the type of egg found in breeding places, only these varieties have appeared. We do not know what proportion of the *maculipennis* positives in Table I are *messeae* and what proportion *typicus*. We have definitely shown, however, that both these varieties are infected in nature. We isolated female *maculipennis* in small containers until they had laid eggs. The egg type was determined and the insect was then dissected. Of 4 positives thus obtained, 3 were *typicus* and consisted of 2 with oöcysts only, and one with sporozoites. One of those with oöcysts had laid eggs of the ‘ pure c ’ type—that is, having no dark areas in the white zone between the two dark bands. The positive *messeae* had sporozoites in the glands. The eggs were typical of *messeae* of a rather dark type. The proportion of positives in the two varieties in this experiment, *messeae* 1 in 147, and *typicus* 3 in 64, has little significance on account of the small numbers and because of the fact that all the positive *typicus* occurred in one collection from the same village (Paradeisos, August, 1932).

The relation of stomach positives to salivary-gland positives varied greatly with the season. *Elutus* will be taken as an example, since the infection rate and the numbers dissected were relatively large in all months. The ratio of the percentages infected, stomach : glands, varied as follows: May and June, 1 : 0·4 respectively; July and August, 1 : 1·0; September and October, 1 : 1·0; November and December, 1 : 1·6. The incidence of oöcysts, then, was highest in the spring and lowest in late autumn and early winter. The

occurrence of sporozoites with no visible remnant of oöcysts was most common in the latter part of the year.

As a routine, we examined dissected mosquitoes with the high dry lens. Nearly all the positives were stained and re-examined, the sporozoites under the oil immersion lens.

Sporozoite rates of different species by months and years. *Elutus*, *maculipennis* and *superpictus* are compared by months and years in Table II. In this Table, only sporozoite rates are shown. We used many of the dissected mosquitoes for the precipitin test of blood meals, and so could not keep collections until the blood had so far digested as to make them suitable for stomach dissections.

An important factor affects the comparison of different years—the great reduction in the blood parasite rate of village children which occurred in this region between the spring of 1932 and the autumn of 1934. Villages in the Plain of Philippi fell from a range of 55-60 per cent. to one of 3-20 per cent.

The reduction was nearly universal, and was due to a drought which diminished the production of *Anopheles*, especially of *A. elutus*. Certain villages in the Plain of Chrysoupolis near the Nestos River were much less affected, but showed some reduction of *Anopheles* in 1934. A diminution in the number of gametocyte carriers, which accompanied the fall in the general parasite rate, naturally affected the sporozoite rate of *Anopheles*.

The dissections of the month of March are represented by a single collection, which is omitted from the Table because it belonged to a special winter study. In this collection, 397 *elutus* gave a sporozoite rate of 0·5 per cent. ; 99 *maculipennis* no positives, and 114 *superpictus* one positive with oöcysts only. We found no positives in April in any locality, although our dissections for that month included large numbers of the more susceptible species collected in highly malarious villages. Wenyon (1921) found oöcyst infection in April among *superpictus* collected in Lahanah Village, Macedonia. In our region, April infections of *Anopheles* must have been rare during the past three years.

Elutus (Table II) in all years shows a fairly steady rise from May or June to midsummer, and a decline in the later months of the year. In 1932, the peak occurred in September ; in 1933, in August ; and in 1934, in July. The numbers dissected in July, 1934, are hardly adequate for a fair comparison, and the percentages positive in August and September of both 1933 and 1934 do not differ greatly. Comparing different years, the rates of the autumn months show a decline which does not appear in those of spring and summer. The summer and autumn of 1932 were warm, while in 1933 and 1934 cool nights began in August or early September and the rest of the summers and autumns were relatively cool. A more important factor is probably the diminution in gametocyte carriers mentioned above. Our blood parasite surveys showed a much greater decline in *P. falciparum*, the prevailing autumn species, than in either *P. vivax* or *P. malariae*.

Superpictus reached its maximum in August in both 1932 and 1933. The

TABLE II

Sporozoite rates of *A. elutus*, *A. maculipennis* and *A. superpictus* for three years, compared by months, all localities

Month	*A. elutus*						*A. maculipennis*						*A. superpictus*					
	1932		1933		1934		1932		1933		1934		1932		1933		1934	
	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive
Jan.			189	2·6					128	0·0					728	0·6		
Feb.			178	1·7					144	0·0					241	0·0		
Mar.																		
April	460	0·0			316	0·0	11	0·0			236	0·0	4	0·0			1	0·0
May	939	0·1	537	0·0	405	0·2	66	0·0	410	0·0	265	0·0	2	0·0	54	0·0		
June	1,715	0·5	1,161	0·1	390	0·8	290	0·0	696	0·1	154	0·0	6	0·0	162	0·6	1	0·0
July	1,723	1·2	2,740	0·7	248	2·0	386	0·5	617	0·0	264	0·0	121	0·8	232	0·4	70	0·0
Aug.	1,588	1·2	2,174	3·0	1,215	1·7	518	0·2	1,549	0·1	810	0·1	577	3·1	877	1·1	15	0·0
Sept.	962	3·8	1,069	2·7	855	1·4	436	0·0	1,406	0·1	473	0·0	952	2·7	776	1·0	672	0·9
Oct.	504	2·0	793	1·1	297	0·3	829	0·2	1,105	0·2	424	0·0	1,189	1·1	937	0·2	491	0·4
Nov.	717	1·5	161	0·0	117	0·0	738	0·0	923	0·0	190	0·0	1,296	1·2	526	0·2	335	0·0
Dec.	389	1·0	138	0·7	220	0·4	503	0·0	580	0·0	562	0·0	1,205	0·4	301	0·7	252	0·0
Total	8,997	1·22	9,140	1·45	4,063	1·08	3,777	0·13	7,558	0·06	3,378	0·03	5,352	1·48	4,834	0·60	1,837	0·44

numbers dissected in midsummer of 1934 are insufficient for comparison. In *maculipennis* the sporozoite rates are too small for satisfactory comparison of seasons. Positives occurred in all months from June to November.

We found the first specimens sporozoite-positive on the following dates: *elutus*, May 21st, 1934; *maculipennis*, June 2nd, 1933; *superpictus*, June 29th, 1933.

The totals of different years, as shown in Table II, are not strictly comparable, because at the beginning of 1933 we extended our territory to include some new villages, among them several highly endemic villages near the Nestos River. To correct this source of error, we have introduced Table III, in which the dissections from the new territory are excluded from the rates of 1933 and 1934. Only totals are given; the seasonal incidence was about the same in the new territory as in the old.

TABLE III

Total sporozoite rates of *A. elutus*, *A. maculipennis* and *A. superpictus* for three years, by years, for only those localities surveyed in all years

	A. elutus			*A. maculipennis*			*A. superpictus*		
	1932	1933	1934	1932	1933	1934	1932	1933	1934
No. examined ...	8,997	6,683	1,180	3,777	4,463	1,555	5,352	4,228	1,778
No. positive ...	110	67	11	5	3	0	79	22	8
Percentage positive	1·22	1·00	0·93	0·13	0·07	0·0	1·48	0·52	0·45
Ratio of indices ...	1·00	0·82	0·76	1·00	0·47	0·0	1·00	0·35	0·30

The comparison of annual rates as corrected for locality (Table III) shows a yearly decline in all three species, less pronounced in *elutus* than in the other two. In *maculipennis* the percentage fell from a very low level to extinction. In both *elutus* and *superpictus* the greatest decline occurred between 1932 and 1933; 1934 showed less change.

In Table IV the number of dissections of *A. algeriensis*, *A. bifurcatus* and *A. hyrcanus* are compared by months. All years and localities are combined. Not a single positive, sporozoite or oöcyst, was found in any of these species. The majority of these dissections were of salivary glands only, but we did many stomach dissections in addition to those mentioned in the note to Table I. The seasonal incidence of dissections corresponds roughly to that of the seasonal densities in all species; specimens available for midsummer dissections were

few. Further comparisons of these species will be deferred to the general comparisons given below.

TABLE IV

Number of dissections of *A. algeriensis*, *A. bifurcatus* and *A. hyrcanus*, all localities and all years combined

	A. algeriensis	*A. bifurcatus*	*A. hyrcanus*
	No. examined	No. examined	No. examined
January ...	0	10	0
February ...	0	0	0
March ...	0	0	0
April	190	10	1
May	14	0	10
June	40	3	6
July	10	4	24
August ...	0	0	6
September ...	152	103	62
October ...	584	320	6
November ...	149	250	0
December ...	15	242	0
Total ...	1,154	942	115

The number of sporozoites among positives. In nearly all sporozoite-positives a record was kept of the number of sporozoites. Numbers were classified in 6 categories, varying from ' very few ' to ' ++++.' In order to get a rough comparison of species, numbers were assigned to each category ; no. 1 to the lowest, no. 2 to the next, and so on up to no. 6. The total number of positives in each category was multiplied by its appropriate value-number, and the sum of the products thus obtained was divided by the total number of positives of a given species. Thus a sort of weighted ' average ' was obtained. The number of positives and the weighted ' average ' of each species were as follows : *elutus*, 337 and 3·0 respectively ; *maculipennis*, 11 and 3·0 ; *superpictus*, 110 and 3·1. Apparently the three species differed little in number of sporozoites.

Anopheline infection rates and locality. In our surveys we visited about 40 different villages at least once during each year ; some of them were visted bi-monthly. We observed great variations in the infection rates of different collections in the same locality. The maximum rate in *superpictus* was observed in September, 1932. Ninety-eight dissections in a single collection gave a total

parasite rate of 10·2 and a sporozoite rate of 5·2. In the same year and month, but in a different locality, 43 *elutus* gave a total rate of 14·0 and a sporozoite rate of 9·3. The variations in different villages were such as to suggest the temporary presence of at least one good gametocyte carrier. We had to ' ration ' our visits to certain localities in order to obtain a fair average for a whole region.

Precipitin tests of blood meals of anophelines. Owing to an improvement in the technique of the precipitin test, which effected a great saving of time and sera, we were able to make a large number of tests of blood meals of anophelines ; over 18,000 positives were obtained during the three years, most of them in 1933 and 1934. In Table V we compare species according to house and stable collections ; all months and years are included, and all localities except one or two screened villages and a few health resorts where domestic animals were scarce. Only those entries are included which gave a positive test for some serum. We tested for man, pig, horse, sheep and cow. About 500 negatives were re-tested for dog and fowl without materially increasing the proportion of positives. We give here only the numbers and percentages positive for man.

TABLE V

Precipitin tests for blood meals, all months from October, 1932, to December, 1934 (positives only ; no cross-reactions are included)

Species of *Anopheles*	Houses			Stables		
	Total positive	Positive for man		Total positive	Positive for man	
		No.	Percentage		No.	Percentage
elutus ...	3,980	2,439	61·3	2,855	213	7·5
maculipennis ...	1,798	381	21·2	4,607	22	0·5
superpictus ...	111	33	29·7	1,611	25	1·6
algeriensis ...	703	111	15·8	2,164	4	0·2
bifurcatus ...	9	1	11·1	139	0	0·0
hyrcanus ...	43	11	25·6	42	0	0·0
All species	6,644	2,976	44·7	11,418	264	2·3

Comment on Table V. We note that *elutus* has a far higher percentage positive for man in both houses and stables than does any other species. *Maculipennis* is much below *elutus* in houses ; in stables the difference is still greater. *Superpictus* is intermediate between these two, but is much closer to *maculipennis* than to *elutus* in both house and stable collections. The ratio of percentages positive for man in these three species in the order *maculipennis* :

superpictus : *elutus* are as follows : houses, 1 : 1·4 : 2·9 ; stables, 1 : 3·2 : 5·0. *Algeriensis* ranks below *maculipennis* in both houses and stables. In *bifurcatus* and *hyrcanus* numbers are inadequate for fair comparison. Both species, or at least *hyrcanus*, tend to be exophilous in this region. The few individuals which remained in villages showed a strong predilection for the blood of domestic animals. The totals for all species show a very small percentage positive for man among stable collections, a percentage which would be insignificant but for *elutus*.

The more detailed analysis of these data by years, months, localities and type of domestic animal must be deferred for other papers ; we include here only such analysis as is especially useful in comparing species with regard to transmission of malaria. The high percentage man-positive among *elutus* appeared in each of the three years during which we made these tests. It was far above either *maculipennis* or *superpictus* in each month of every year in which numbers were adequate for comparison. During the months of least divergence it was well above *superpictus* and far above *maculipennis*. The only localities in which *maculipennis* showed relatively high human incidence were certain localities where domestic animals were few. Nearly all the positive *algeriensis* came in the spring and autumn months. In all positives, including those of man and domestic animals, over 60 per cent. gave a reaction for the cow. Even in *elutus* (including those from both houses and stables) more cow-positives were found than man-positives ; we can only say that the deviation to domestic animals is less marked in *elutus* than in any other species.

The character of the blood meals of sporozoite-infected Anopheles. During the past two years we have dissected many *Anopheles* soon after collection or have kept them on ice to prevent digestion of blood until examination. Among those with stomach blood fresh enough for a precipitin reaction, we found 61 with sporozoites in the salivary glands. Nearly all these were found during the period July to October inclusive, the majority of them in midsummer. These dissections give us a record of the last meal taken by an infected anopheline. The results appear in Table VI.

These numbers (Table VI) are not very large, but they indicate one thing clearly—the value of domestic animals in the deviation of infected anophelines from man. Again, they afford a strong presumption that the feeding habits of infected *elutus* do not differ greatly from those of *elutus* in general. The percentage of these infected *elutus* positive for human blood meals is very near that of all *elutus* in midsummer. The percentage of the sporozoite-infected positive for cow blood meals, 57·4, is comparable with that of cow blood positives in all *elutus*. Apparently infected *elutus* does not differ greatly from *elutus* generally in regard to choice of host.

The numbers of *maculipennis* and *elutus* in Table VI are too small for any generalization ; they indicate the same high predilection for domestic animals as these species do generally.

We hope to enlarge this list of sporozoite carriers, precipitin-positive, and to include some determinations of the maxillary index.

The number and kind of domestic animals in villages. We obtained from the municipal census of 1934 the number of domestic animals in 16 villages situated in the region where mosquitoes were collected for the precipitin tests. Among approximately 11,000 inhabitants the number of domestic animals per 100 persons are : horses and donkeys, 10 ; cattle, including buffalo, 50 ; sheep and goats, 100 ; pigs, 7. These proportions varied greatly in different localities, depending on the amount of pasturage available and on other factors.

Table VI

The character of blood meals in sporozoite-infected *Anopheles.* Host, place of collection and character of blood meals ; numbers and percentage incidence

Species	Host	Houses		Stables	
		No.	Percentage incidence	No.	Percentage incidence
A. elutus	Man... ...	19	47·5	1	7·1
	Horse ...	1	2·5	1	7·1
	Cow ...	19	47·5	12	85·7
	Man × Cow	1	2·5		
	Total ...	40	100·0	14	99·9
A. maculipennis	Horse ...	1		—	—
	Cow ...	—	—	1	
A. superpictus	Man... ...	0		1	20·0
	Cow ...	5		4	80·0
	Total ...	—	—	5	100·0

Of most importance in malaria transmission is the number and kind of animals present in villages at night during the summer. We made a house-to-house night census of two villages where the principal species of *Anopheles* are *maculipennis* and *elutus*, and where malaria is very prevalent. Including only people and animals present in the villages during the nights of July 26th to 28th, 1934, the number per 100 inhabitants were : horses, 44 ; cattle, including buffalo, 120 ; pigs, 6. Twenty-nine per cent. of the people were sleeping out-of-doors and 96 per cent. of the domestic animals spent the night outside the stables.

Comparison of houses and stables as daytime resting-places. In Table VII, the average number per collection in houses and stables is shown for all species. We compare only the collections of 1934, which are fairly representative of those of all years. The collections in a single room, generally a bed-chamber, count as one house collection unit ; a stable room or compartment, as one stable unit. The stable room is usually larger than that of the house, and collections in stables with high, thatched roofs are usually less complete. We collected all species indiscriminately, however, so that the species-composition in the two types of resting-place may be compared. In winter, nearly all species tend to collect in stables ; the averages of Table VII represent essentially those of the warmer months. The number of collections is not the same for all species, since we included for the most part only collections in localities where the respective species were fairly abundant. *Bifurcatus* and *hyrcanus* were relatively scarce and occurred in many localities. In these two we divide the numbers collected

TABLE VII

Incidence of species in houses and stables

Species of *Anopheles*	Houses			Stables			Ratio of averages house : stable
	No. of collections	No. collected	Average per collection	No. of collections	No. collected	Average per collection	
elutus ...	1,166	4,351	3·7	508	6,479	12·7	1 : 3·4
maculipennis	1,166	3,742	3·2	508	10,612	20·9	1 : 6·5
superpictus	336	432	1·3	262	6,821	26·0	1 : 20·0
algeriensis	390	467	1·2	270	2,294	8·5	1 : 7·1
bifurcatus...	2,889	27	0·01	1,818	244	0·1	1 : 10·0
hyrcanus ...	2,889	97	0·03	1,818	110	0·06	1 : 2·0

by the total visits of the season, which gives only an approximation. A reasonably accurate comparison of *elutus* and *maculipennis* is possible, since these species were abundant in the same plain at the same season.

We may assemble our data in another way. Of the total number collected during the year the following percentages were found in houses : *elutus*, 40·2 ; *maculipennis*, 26·0 ; *superpictus*, 6·0 ; *algeriensis*, 16·9 ; *hyrcanus*, 46·8. These percentages and the averages of Table VII permit a fair comparison of the four commoner species : *elutus* gives the highest degree of preference for houses ; *superpictus*, for stables ; *maculipennis* and *algeriensis* are intermediate between these two.

We compare, of course, only mosquitoes remaining in villages during the daytime. It may be that the majority of some species prefer to remain out-of-doors, choosing neither houses nor stables for daytime refuge, especially during

the warmer months. We have frequently found adult anophelines of several of the species in hollow trees, wells, cavities in the banks of streams and elsewhere in the open. Such places rarely contained large numbers, except when the total density was also large ; they were always poor collecting places as compared with houses or stables.

Elutus is the only species in which house-frequenting, sporozoite index, and percentage human-positive of blood meals run at all parallel. *Maculipennis* ranks next to *elutus* as a house-frequenter, but has a much lower sporozoite and human-positive precipitin percentage. *Superpictus*, judged by these standards, is more disposed than is any other species to feed in houses and to fly to stables for daytime shelter.

II. INFECTION IN THE LABORATORY

In our laboratory experiments we compared different anopheline species with respect to their susceptibility to malaria. We are well aware of the sources of error in such experiments. The same species of mosquito will bite freely at one time and refuse at another, depending often on the condition of the specimens at the time of exposure to the gametocyte carrier. A gametocyte carrier will infect a large proportion of a species on one day and few or none on the next. A promising carrier with many gametocytes may not infect a single mosquito. Adequate numbers are important ; a few batches prove little.

In order to minimize these sources of error we have followed certain rules. All mosquitoes were fed but once on a carrier, and only those which took blood were preserved for dissection. Fully engorged specimens and those partly engorged were, for the most part, kept separate ; the proportion of positives in the latter group was smaller. In estimating the percentages infected, only those batches were included in which at least one positive occurred in some species on the day of feeding, thus showing that the gametocyte carrier harboured viable gametocytes on that day. A partly engorged batch was included only when a positive appeared in it, whatever the result of fully engorged batches of the same day. Very few of such partly engorged batches were included.

Of batches which fulfilled these conditions we obtained approximately 16 in 1932, 45 in 1933, and 7 in 1934. Experiments were undertaken in different years whenever promising gametocyte carriers and anophelines were available. The great majority of anophelines tested had been bred out in the laboratory from larvae or pupae collected in their natural breeding places ; a few were reared in the laboratory from eggs. A small number were collected in the adult stage ; in these we had to distinguish laboratory infection from a possible natural infection by the size of oöcysts.

The results of the three years' experiments are given by species in Table VIII.

TABLE VIII

The susceptibility to malaria of different species of *Anopheles*, as shown by laboratory experiments

Year	*A. elutus*		*A. maculipennis*		*A. superpictus*		*A. hyrcanus*		*A. algeriensis*		*A. bifurcatus*	
	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive
1932	143	21·7	31	19·3	39	51·3	0	0·0	0	0·0	0	0·0
1933	76	17·1	58	20·7	68	55·9	6	0·0	7	85·7	1	100·0
1934	12	25·0	15	20·0	9	66·7	1	0·0	0	0·0	0	0·0
All years	231	20·3	104	20·2	116	55·2	7	0·0	7	85·7	1	100·0

Comment on Table VIII. Of the three commoner species, *elutus*, *maculipennis* and *superpictus*, *superpictus* is apparently the most susceptible by far, and *elutus* and *maculipennis* about equal. This relationship comes out in each of the three years and is probably significant of the relative susceptibility of these species in nature. Little is indicated by the small numbers in the other species further than that *algeriensis* and *bifurcatus* may be infected in the laboratory. *Algeriensis* showed large numbers of oöcysts ; *bifurcatus*, only 2 ; both species were infected with *P. falciparum*. Positives were obtained in *elutus*, *maculipennis* and *superpictus* with both *P. vivax* and *P. falciparum*. Sporozoites were obtained in all three with *P. falciparum*, and in *elutus* and *superpictus* with *P. vivax* also. We obtained *P. vivax* sporozoites in oöcysts of *maculipennis* but none in the glands, probably only because too few infected specimens were kept long enough fully to ripen sporozoites.

Summary and discussion of the evidence regarding the transmission of malaria by Macedonian Anopheles, *by species. Algeriensis* is easily infected in the laboratory with *P. falciparum.* It is very plentiful in some seasons and is frequently found in houses. The Sergents (1905) have found it sporozoite-infected in nature in Algiers. In this region we have not found a single infection in nature, sporozoite or oöcyst, among over 1,100 dissected, although many were collected in villages which had infected *elutus* at the same time. It will bite man freely in the open, but the percentage with stomach blood human-positive among collections in houses and stables is comparatively low. It is comparatively rare in midsummer. Villages in which it is most plentiful have shown no infants infected during 1933 and 1934. In Macedonia it is probably negligible

as a vector of malaria, at least during seasons such as we have had during the past three years. It is noteworthy that among thousands of this species collected in houses and stables not a single male has appeared.

Bifurcatus and *hyrcanus* also are probably of little importance in this region. Neither is much inclined to visit human dwellings or stables, and neither has shown a single infected specimen in nature. Both give a low percentage positive for man in the precipitin test. *Bifurcatus* was infected in the laboratory (oöcysts with *P. falciparum*) and is considered an important carrier in certain large cities. In this region it is comparatively rare in midsummer. *Hyrcanus* occurs in the warm season ; few are found in houses or stables.

Elutus is plentiful throughout the summer and common in human dwellings. the percentage found infected in nature has been relatively high in all seasons and has shown but little diminution through three years. It is readily infected in the laboratory with both *P. vivax* and *P. falciparum.* It shows far less deviation to domestic animals than does any other species. Our experiments with batches stained and released has shown a survival, in midsummer and under natural conditions, of 26 days. Its flight-range in midsummer, determined by recovery of stained specimens and by its density in villages distant from the breeding place, is at least 4½ km. and in effective numbers.

Maculipennis is similar to *elutus* in several respects. It enjoys a seasonal prevalence equally long and an ability to survive in midsummer under natural conditions (at least 26 days in our experiments) likewise far beyond the period necessary for the maturation of sporozoites. It also occurs in large numbers and is abundant in human dwellings. It is apparently equally susceptible to malarial infection, as shown by laboratory experiments. As regards deviation to domestic animals and infection (both with oöcysts and sporozoites) in nature, *maculipennis* diverges sharply from *elutus.* The difference in the sporozoite rates of these two species has been shown in the foregoing Tables. This difference is even more definitely shown by collections in which both species were found on the same day in the same house or stable (in all of 1934 and the most of 1933 in the same room), and in which at least one sporozoite-positive appeared in some species, showing the presence, then or recently, of an effective gametocyte carrier in the neighbourhood. In 1933 we had 71 such collections, 32 in houses and 39 in stables, comprising in all 1,095 *elutus* and 1,009 *maculipennis.*

The sporozoite rate of *elutus* was 7·67, that of *maculipennis*, 0·29, the ratio of percentages being 26·1 : 1. In 1934 we had 25 collections which fulfilled these conditions—15 in houses and 10 in stables, comprising in both 362 *elutus* and 302 *maculipennis.* The sporozoite rate of *elutus* was 7·73, that of *maculipennis.* 0·33, the ratio of percentages being 23·4 : 1. These collections were made during the periods June to December, 1933, and May to December, 1934 ; the great majority occurred in July, August and September of both years.

Apparently deviation to domestic animals best explains the difference in the sporozoite rates of these two species. *Maculipennis* in our region, where

domestic animals are plentiful, is probably of little or no practical importance in the transmission of malaria ; certainly not during the past two years. The sporozoite rate, very low in 1932, fell almost to zero in 1934.

Superpictus is very plentiful in some parts of this region, but reaches its maximum density somewhat later in the season than does either *elutus* or *maculipennis*. It is apparently very susceptible to malaria, for its infection rate in the laboratory was nearly 3 times that of *elutus* or *maculipennis*. In nature, its sporozoite rate was above that of *elutus* in 1932 ; in 1933 it dropped to a low level and further decreased in 1934. The percentage human-positive in the precipitin test is somewhat above that of *maculipennis* but far below that of *elutus*. It occurs in human dwellings, but is more disposed to rest in stables than either *elutus* or *maculipennis*. Degeneration of sporozoites is more pronounced in *superpictus* than in the other species. This factor is difficult to measure and will be discussed more fully in a subsequent paper.

The rôle played by *superpictus* in this part of Macedonia is difficult to determine. Wenyon (1921), working in the region near and east of Salonika, found this species infected in nature. He also infected it in the laboratory and considered it an important vector of malaria, especially in the hill region.

At the end of our work in 1932, we were persuaded that it is of much importance in this region also ; but the work of subsequent years has led us to re-open the question. The matter is an important one in the combat against malaria, for the breeding places of *superpictus* are usually quite different from those of *elutus* and are sometimes especially hard to cope with. One would be glad of evidence which would justify the limiting of the attack to *elutus* alone. This consideration may excuse a rather detailed discussion of the importance of this species.

We do not have in the region covered by our survey a locality in which *superpictus* is not associated with *elutus*, so that we cannot solve our problem by elimination. During the past three years, however, meteorological conditions have been such as greatly to reduce the production of *elutus*, while *superpictus*, a breeder in spring-fed streams, has been less affected by drought. As a result, we have villages in which the *superpictus* density has remained high while that of *elutus* has become low (in 1934 almost nil after July). Both the sporozoite rate of *superpictus* and the malaria parasite rate among village children has greatly fallen in these villages. We describe two or three of these villages in more detail.

The villages Lefki Alpha and Lefki Beta are only about 1 km. apart and are both situated on a permanent stream very productive of *superpictus*, so that they may be fairly treated as a unit. Kara Orman is 5 or 6 km. distant from the Lefkis, but receives its *elutus* from the same source. A series of some five or six mountain streams immediately above the village affords a permanent supply of *superpictus*. In Table IX we compare in the three villages the sporozoite rates of *elutus* and *superpictus* and the parasite rates for 1932, 1933 and 1934.

TABLE IX*

Sporozoite rates of anopheline species and parasite rates of village children, compared by years

I. *Lefki A and B*

Dissections of *Anopheles*

1932				1933				1934			
A. elutus		*A. superpictus*		*A. elutus*		*A. superpictus*		*A. elutus*		*A. superpictus*	
No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive
455	1·3	1,460	1·1	225	0·9	1,121	0·7	1	0·0	594	3·0

Parasite rates of village children

1932			1933			1934		
No. examined	No. positive	Percentage positive	No. examined	No. positive	Percentage positive	No. examined	No. positive	Percentage positive
82	43	52·4	116	28	24·1	114	12	10·5

II. *Kara Orman*

Dissections of *Anopheles*

1932				1933				1934			
A. elutus		*A. superpictus*		*A. elutus*		*A. superpictus*		*A. elutus*		*A. superpictus*	
No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive	No. examined	Percentage positive
163	1·2	1,406	1·8	543	0·2	1,511	0·3	4	0·0	885	0·6

Parasite rates of village children

1932			1933			1934		
No. examined	No. positive	Percentage positive	No. examined	No. positive	Percentage positive	No. examined	No. positive	Percentage positive
44	34	77·3	53	17	32·1	52	5	9·6

* In Lefki Alpha and Beta 208 *maculipennis* were dissected in all years ; in Kara Orman, 146. All were negative.

The numbers dissected (Table IX) only approximately represent the density of these species during the three years. *Superpictus* was plentiful throughout the summers, but the density of *elutus* varied with the life of the breeding place, which was about one month shorter in each succeeding year. It was high until September in 1932, until August in 1933, but only until July in 1934. The probable course of events in these villages was as follows. In 1932 *elutus* and malaria were very prevalent. The numbers of overwintering *elutus* found in the spring of 1932 and the high parasite rates of that spring would support that conclusion. The density of *elutus* diminished to a low level by 1934, while that of *superpictus* remained high ; the parasite rate of village children greatly diminished. The sporozoite rate of both species diminished, that of *superpictus* to a greater degree. The numbers of *elutus* dissected are inadequate for a fair comparison. At all events, it appears that *superpictus* alone was unable to maintain a high malaria prevalence in 1934, in spite of the abundance of gametocyte carriers present in the earlier years.

The parasite rates of young children found in these villages in the autumn survey of 1934 is significant. Among 9 infants 12 months old or under, and 14 children $1\frac{1}{2}$ to 3 years of age, not one positive appeared.

Two or three other villages also showed a pronounced fall in the parasite rate in spite of the presence of *superpictus*, but in these the *superpictus* density was much lower than in the three villages described.

It is quite possible that a species which, like *superpictus*, has a relatively low human precipitin rate may show many infections by virtue of the large number of gametocyte carriers provided by a more effective associated species, and without this powerful ally it might show but a small oöcyst or sporozoite rate. *A. pharoensis* is not considered a dangerous species in Lower Egypt.* In Southern Nigeria, Barber and Olinger (1931) found a sporozoite rate of 0·7 per cent. among 281 dissected of this species. But it was associated with numerous *A. costalis* with a sporozoite rate of 6·6 per cent. It is probable that *pharoensis* alone might have shown an insignificant amount of infection. Unfortunately, we do not know the percentage of *pharoensis* positive for human blood meals ; that of *costalis* is nearly 100 per cent. It is possible that the rare cases of *subpictus* infected in nature may be due to associations with *sundaicus* (*ludlowi*) or some other good vector.

Superpictus has such a generally bad reputation that much evidence is required to exculpate it even partially. Our evidence is based on the observations of one region and of only three years, the last two of which had relatively short warm seasons. During a very long warm season here or in a warmer region, *superpictus* might become more dangerous ; and, in a region less abundantly supplied with domestic animals, both *maculipennis* and *superpictus*

*Henderson (1932) considered this species as a probable carrier in the upper Nile Province of Sudan ; no dissections were recorded, and it was associated with *funestus* and *gambiae*.

might be important vectors.* We believe, however, that our work has established a presumption sufficiently strong to warrant the attack on *elutus* only in this region. At all events, it would be worth a trial.

Animal parasites other than malaria. In the course of our routine dissections we have made a practice of noting all animal parasites. We have not attempted any detailed study of their morphology or taxonomy; but, since the series of observations is a large one, extending over two years and including many thousands of dissections, we include here some data on the general types of parasite and their incidence in various seasons and species of *Anopheles*.

The commonest parasites were round worm, flat worm, flagellates including *Crithridia fasciculata*, and various *Microsporidia* which we do not attempt to classify. Of the latter, one form much resembles the *Thelohania* common in larvae of *Anopheles*. This form usually attacks cells within the stomach; free clumps of cells are also common in the thorax. Another form appeared as short, curved, sausage-shaped bodies, often in cysts in the salivary glands and on the surface of the stomach, where in some stages they superficially resemble oöcysts of malaria parasites. Forms like the ' sausage ' type, but not curved, were common, sometimes occurring in cysts in or along the muscles of the stomach.

The *Thelohania*-like *Microsporidion* was commoner in the anterior part of the stomach, the flagellates in the posterior, often invading the lower gut and the Malphighian tubules. Round worms were more often within the tubules, which they enlarged and distorted, and within the stomach; flat worms were more commonly found on the exterior of the stomach wall. Both kinds of worm were frequently found in the thorax. The *Thelohania*-like *Microsporidion* and the flagellates often caused extensive damage to the tissues of the mosquitoes; the other parasites, apparently, were as harmless as are malaria oöcysts to the cells of the stomach. We do not know to what extent these parasites shorten the life of the mosquito, but they probably do not prevent the transmission of malaria, for we have occasionally found both round and flat worms associated with sporozoites of malaria parasites. In one or two cases, oöcysts of malaria were found on the posterior part of a stomach and an extensive invasion of the *Thelohania*-like *Microsporidion* within the anterior part.

Of different species of *Anopheles*, *maculipennis* was more often attacked by these non-malarial animal parasites than any other species; *superpictus* was least often attacked. Among the principal *Anopheles* the totals were: *elutus*, 15,450 dissected, 2·4 per cent. infected, all parasites; *maculipennis*, 10,435 and 4·1

* In a recent malaria survey of Cyprus (July, 1935), we found very high blood parasite and spleen rates in certain villages in which *superpictus* was apparently the only vector. The sporozoite rate of this species (1,134 dissected) was 7·8 per cent., and the percentage human positive of blood meals (315 positives, houses and stables combined) was 18·7, a precipitin rate 5 or 6 times greater than that found in Macedonia. In Cyprus summers are long and hot, domestic animals in villages at night are relatively few, and a large percentage of both villagers and their animals sleep out of doors at night.

respectively ; *superpictus*, 7,342 and 0·2. *Maculipennis* is somewhat less susceptible to malaria than is *superpictus*, as indicated by our laboratory infection experiments. Probably the breeding places of the different species are of importance. Larvae of *elutus* and *maculipennis* are usually found in more stagnant water than are those of *superpictus*, which prefer spring-fed streams and pools.

Certain localities have shown a much higher percentage than others of these non-malarial parasites. The village of Peristereon, dissections of 1934, will be given as an example, collections from all other localities (1934) serving as a control. *Elutus*, Peristereon (766 dissections) and controls (4,257 dissections) gave the following percentages in the locality order given : round worms, 0·5 and 0·4 ; flat worms, 2·1 and 0·3 ; flagellates, 0·3 and 0·02 ; *Microsporidia*, 2·6 and 0·8. *Maculipennis*, Peristereon (1,001 dissections), controls (2,409 dissections) : round worms, 0·5 and 0·3 ; flat worms, 1·5 and 0·5 ; flagellates, 0·6 and 0·08 ; *Microsporidia*, 2·9 and 1·1. The numbers dissected are so large that even small differences in percentages are significant. During that year at Peristereon, *elutus* and *maculipennis* bred in stagnant pools, mostly in or near woods.

In eight or nine instances we have found these parasites in mosquitoes bred out in the laboratory and fed on malaria gametocyte carriers. The list includes : flat worm, 3 times in *maculipennis*, once in *elutus* ; *Microsporidia*, twice in *maculipennis* and 4 times in *elutus*. The *Microsporidia* included both the *Thelohania*-like and the ' sausage ' forms. The parasites were probably in the larvae or pupae when collected in natural breeding places. They had received no blood except that of man.

We have only one noteworthy instance of the predilection of any parasite for a species of *Anopheles* : flat worm occurred in *maculipennis* in 1·8 per cent., round worm in only 0·35. The percentages in *elutus* were : flat worm, 0·54 per cent., round worm, 0·68. *Superpictus* had insignificant numbers of either parasite.

Round worm showed a marked seasonal incidence. It was very common in the summer, but rare in winter and then often in a quiescent stage. The other parasites were common during the cold months of the year.

We found a few infections with a trypanosome-like flagellate. Spirochaetes occurred occasionally. Among plant parasites, bacteria in living *Anopheles* were very common. Salivary glands of *Anopheles* were often deformed by bacteria or cysts of *Microsporidia* ; they were sometimes deformed by sporozoites, as noted by Giovannola (1933), but not always so when sporozoites were few and localized.

The acicular crystals described by Wenyon (1921) were commoner by far in *maculipennis* than in any other species. These crystals might be mistaken for sporozoites by the unwary, especially when suspected positives are not as a routine stained and examined under the oil immersion. These crystals were not noted by us among thousands of *A. costalis* dissected in West Africa.

SUMMARY

This paper describes a study made during the years 1932, 1933 and 1934 of the relation to malaria of the 6 species of *Anopheles* common in a region of East Macedonia. It appears that *A. elutus* is the chief vector of malaria—possibly the only important one in this region during the past three years. Two other species, *A. maculipennis* (including both the local varieties, *messeae* and *typicus*) and *A. superpictus*, were also found infected in nature. The sporozoite rate of the first was very low, and epidemiological evidence indicates that the rôle of the second in the transmission of malaria has been unimportant, at least during the past two years. Both of these species might be important carriers in regions with warmer climate or fewer domestic animals. The sporozoite rates of all species have declined during the past three years—a decline associated with diminishing parasite rates in the village populations. A diminished rainfall, affecting especially the density of *A. elutus*, seems to be responsible for the fall in the parasite and the associated gametocyte rates of the populations. Susceptibility to malarial infection, as shown by laboratory experiments, is highest in *superpictus* ; *elutus* and *maculipennis* are about equally susceptible. Deviation to domestic animals, as determined by precipitin tests of blood meals, was far less pronounced in *elutus* than in any other species. The precipitin test of *elutus* found infected in nature indicates a deviation to domestic animals comparable with that of non-infected *elutus*. *A. algeriensis* and *A. bifurcatus* were infected in the laboratory with *P. falciparum*, but neither of these species nor *A. hyrcanus* were found infected in nature. With regard to choice of daytime resting places, *elutus* shows the highest house preference of any species ; *superpictus*, the lowest. A summary of observations on animal parasites other than malaria is given.

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AN INQUIRY INTO VITAMIN A DEFICIENCY AMONG THE POPULATION OF TESO, UGANDA, WITH SPECIAL REFERENCE TO SCHOOL-CHILDREN

BY

L. J. A. LOEWENTHAL, M.D., M.R.C.P., D.T.M. & H.

(*Uganda Medical Service*)

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CONTENTS

I. INTRODUCTION

In previous papers, my colleagues, Mitchell (1933) and Owen (1933), and I (1933) described the occurrence of vitamin A deficiency among prisoners whose diet was adequate in other respects. In these papers were mentioned three free individuals, inhabitants of Buganda Province, Uganda, who showed signs of this deficiency. One had advanced disease of the liver ; the second had lived on cassava for long periods from financial reasons ; and the third was a mental case from whom no history could be elicited, but who had presumably been under-nourished as the result of his condition.

Among thousands of other members of the free population of Buganda we saw no further cases, although we were constantly watching for signs of the deficiency in the Eye and Skin Departments. We attributed this freedom from vitamin A deficiency to the eating by the general population of large amounts of plantain and sweet potato, known sources of vitamin A.

When, in Nairobi in 1932, I demonstrated prisoners showing the follicular eruption typical of the deficiency, Dr. J. H. Sequeira remarked that he had

seen numerous cases of this dermatosis among the free population of parts of Kenya. This is, to a large extent, a grain-eating community ; and on my appointment to Teso District, Uganda, in 1934, I was naturally eager to find out whether vitamin A deficiency was a condition prevalent among the grain-eating Iteso. During the first few months, at the end of the rainy season, a few sporadic cases of xerophthalmia and phrynoderma* were seen. In the month of April, however, after 3 months' dry, hot weather with only occasional rain, the deficiency became prevalent among the children of the district. The manager of one of the elementary vernacular schools (Assamuk) asked my advice about an epidemic of ' scabies ' which had been prevalent during the previous weeks ; suspecting phrynoderma, I examined the pupils and found that 47·5 per cent. of them showed symptoms or signs of vitamin A deficiency. Other schools were then visited systematically until 1,000 pupils had been examined; in addition, 26 prisoners from a county goal and 86 adult members of the free population were examined for purposes of comparison. The results of the examination of 1,112 individuals from different parts of Teso form the subject of this paper.

The Iteso number about 300,000. They are an ' Hamitic-speaking ' people, whose language is very much akin to that of their neighbours the Karamojong, and of the Turkana and Masai. Like these, they are cattle-owners, but instead of living principally on meat, blood and milk, as do the others, they subsist on crops of various kinds, supplemented with milk and, very occasionally, meat. Cattle are a standard of wealth and are not readily disposed of either by sale or slaughter. Cotton is now grown in many parts of Teso, but the tendency is for the peasant to grow only sufficient for his cash needs (i.e., for taxation) ; it is not uncommon to see half-picked cotton fields, enough having been marketed for the year's tax and the rest allowed to go to waste.

In stature the Etesot is tall and lanky ; in temperament he is cheerful and industrious up to a point. The point is reached when his food and drink seem assured until the next crop, and no provision is made for adverse circumstances. In recent years ploughs and clothes have been introduced into Teso, but the naked peasant, with his wives, cattle and primitive instruments of cultivation, is still a feature of the district.

II. FACTORS AFFECTING THE DIET

(*a*) *Geographical* (see map)

Teso District comprises 4,052 square miles of the northern part of Uganda's eastern province. On the south and west it borders on fertile country and lake shore ; to the north-east lies the district of Karamoja, thorn scrub country with absolute drought for over 6 months in the year. Teso may

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*The name given to the follicular dermatosis of vitamin A deficiency by Lucius Nicholls of Ceylon (1933).

conveniently be divided by a line drawn from north-west to south-east, the part below this line being fairly fertile, that above dry, hot and indifferently watered.

(*b*) *Climatic and Seasonal*

The dry season begins about the middle of December and lasts until the end of March. During this time the aspect of the country changes from green to

MAP OF TESO.

brown, hot winds blow daily, and many of the rural water-supplies dry up. The north-eastern part of the district shows these features more markedly than the south-west, and daily shade temperatures of 92° to 98° Fahrenheit are the rule. Occasional rain falls in January and February, but is not usually sufficient to bring on crops, and if such crops appear ensuing dry weather

frequently kills them. The rainfall for Teso is given in the accompanying Table.

TABLE I

Rainfall in Teso, 1934-35

	1934	1935
January	0·0	0·0
February ...	0·17	1·65
March	1·91	1·15
April	7·24	
May	3·60	
June	6·55	
July	6·00	
August	6·07	
September ...	2·57	
October... ...	6·02	
November ...	1·02	
December ...	1·80	

During the first three months of the year, cattle feed on the brown standing grass, where it has not been burnt off as a clearing measure. They become thin and their yield of milk diminishes; although estimations have not been made in Teso, it is reasonable to assume that the vitamin A content of the cow's milk is also reduced, a state of affairs comparable with that obtaining among cattle in Europe on 'winter feed.'

(*c*) *Crops*

Millet of various kinds is grown throughout Teso; some is stored for the dry season, pounded into flour ('atap'), boiled and eaten. Other standard vegetable foods are cassava and maize meal. During the greater part of the year the diet is improved by additions of sweet potato and green vegetables; in the dry season, however, these are not available, and, although it is customary to dry sweet potatoes and store them for the dry months, in bad years such as 1934 this is not done to any extent.

Ground-nuts take the place of these adjuvants during the dry season; although they contain about 50 per cent. of oil by bulk they are not a source of vitamin A.

It will be observed from the foregoing that the vegetable diet during the months of January, February and March is grossly deficient in vitamins A, B_1, B_2 and C.

(*d*) *Foods of Animal Origin*

What milk is obtained in the dry season is rarely drunk fresh; a few natives drink it boiled and a few others take small quantities in tea, but the

great majority allow it to sour. The curd is then eaten and the whey is often mixed with the solid food. In many families, however, milk is not drunk at all; in others the curd of the soured milk is sold; and in the special case of the Iteso living near Assamuk, the whole milk is sold either to the Government creamery or to the Indian population of Kuju (Amuria). The majority of school-children (non-boarders) state that they drink milk twice weekly; often this is whey only, and in a few of the wealthier families it consists of a few teaspoonsful of milk in a cup of tea.

Eggs are eaten (about two a week) by inhabitants of the north-eastern area; they are always cooked first and are never eaten by women.* Meat is is eaten so rarely by all but the wealthiest that it need not be considered as an article of diet. Fish is unobtainable by natives except on the lake shore. Rats are not eaten by either sex.

(*e*) *Native Beer*

Native beer is drunk by all natives almost daily, from infancy onwards. It is customary to start the day in Teso households by drinking a cup or gourd of beer; food is not partaken of until noon, when the serious work of the day is usually over. The beer is prepared from millet and is fermented by yeasts. I believe that its importance as a pellagra- and scurvy-preventive is considerable. After an exceptionally bad millet-year beer may fail, and it will be interesting to note whether deficiencies other than that of vitamin A make their appearance.

It will be seen, then, that during the dry season the population lives chiefly on grain, cassava, ground-nuts and beer, foodstuffs totally or almost totally deficient in vitamin A.

III. SCOPE OF THE INQUIRY

The investigation deals primarily with the schools, of which there are several kinds: they may be classed as bush schools (B.S.), elementary vernacular schools (E.V.) and teachers' training schools (T.T.S.). In the first category are found children of all ages, and even adults, as the teaching often includes religious instruction as a preparation for baptism; the E.V. schools contain pupils from the ages of 6 to 20; and in the last category are adults only. There are no boarders among bush-school pupils, and in the other types of school the boarders' diet is precisely the diet of the surrounding natives, with the exception of milk, which is not supplied in schools. Many boarders, however, go home at week-ends and obtain a certain small quantity of milk then. Fees vary in the different schools, and, where two schools of the same grade are near to each other, it is found that the sons of the wealthy classes patronize the more expensive establishment.

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*A native ' tabu ' common in tropical Africa.

The inmates of 15 schools were examined, these schools being situated in four areas, Amuria, Toroma, Serere and Ngora. At each school the diet of the surrounding natives was ascertained, and modifying circumstances, such as the social and financial position of the pupils' parents, were inquired into. The pupils in 5 schools were divided into full adults, adolescents, and those under puberty, and each group was then examined for the signs of vitamin A deficiency. One thousand school inmates were examined in all.

For purposes of comparison 86 adult natives were examined at Toroma; they were apparently free from signs of other disease. In addition, 26 prisoners in the county gaol at Amuria were examined.

IV. STANDARDS OF VITAMIN A DEFICIENCY

Xerophthalmia not due to a local cause, and the follicular eruption (phrynoderma) described by Frazier and Hu (1931), Nicholls (1933) and myself (1933) were taken as criteria of vitamin A deficiency. It should be noted, however, that general dryness of the skin is an earlier sign than the appearance of phrynoderma; over 80 per cent. of school-children suffered from this dryness at the time of examination, but they were not included as positive cases unless they showed xerophthalmia or the follicular eruption as well. I believe, nevertheless, that this dryness of the skin, which the natives regard as a manifestation of the dry season, is itself the earliest sign of vitamin A deficiency. The follicular eruption varies with the age of the child; in the very young it may resemble 'goose-flesh,' while the elder children show small papules, and the adults the typical black follicular lesions described by the authors quoted above.

No case of keratomalacia was seen, but night-blindness was complained of frequently. A subjective symptom, however, is of little value when dealing with large numbers of cases; in the youngest children tests would have to be carried out, and in the older pupils very careful questioning would have been necessary before specifying a person as night-blind or endowed with normal vision. Even had time permitted, the results of such a course might have been fallible in a community not gifted with the power of observation. Night-blindness was not, therefore, taken as a criterion of vitamin A deficiency after the examination of the first school (Assamuk); had it been included, the percentage of vitamin A deficients would undoubtedly have been higher in the other schools.

Facial acne was common both among those suffering from vitamin A deficiency and among those not so affected. It was not taken as a sign of such deficiency in compiling these records, but it is noteworthy that its incidence among the vitamin A deficient pupils over puberty in Assamuk School was 75 per cent., while it was only 50 per cent. among the non-deficient pupils over puberty at the same school.

V. DETAILED RESULTS

(The examinations were made during the second and third weeks in April.)

1. Assamuk School. 80 pupils.

This has the highest percentage of deficiency cases. The reasons for this are :—

(*a*) The school was first opened this year and has a large proportion of young pupils.

(*b*) The pupils are drawn from a poor class.

(*c*) Night-blindness was included as a criterion of vitamin A deficiency.

(*d*) Many of the natives living in Assamuk sell milk to the creamery and to Indians at Kuju.

Table II

	Over puberty	Under puberty	Total
Total examined...	48	32	80
No. with xerophthalmia	3	4	7
No. night-blind...	12	17	29
No. with phrynoderma	14	12	26
No. with acne	29	0	29
No. with one or more signs of vitamin A deficiency (not acne only)	20	18	38

Comment. It will be noted that acne does not occur in vitamin A deficiency before puberty.

The incidence of vitamin A deficiency is somewhat higher in the younger pupils. Most of those in the ' over puberty ' group, however, were approximately only 14 or 15 years old, otherwise the difference would have been still more marked.

2. Elementary Vernacular School, Serere. 53 pupils.

Table III

	Boarders	Non-boarders	Total
Total examined...	15	38	53
No. with xerophthalmia	2	3	5
No. with phrynoderma	5	11	16
No. with one or more signs of vitamin A deficiency	5	13	18

Comment. The incidence of vitamin A deficiency is precisely the same in the two groups. Similar results were obtained at the other boarding-schools.

Table IV

	Over puberty	Under puberty	Total
No. examined	20	33	53
No. with signs of vitamin A deficiency ...	1	17	18

Comment. The difference in these groups is very significant.

3. Bush School (R.C.), Ngora. 128 boys.

Table V

	Over puberty	Under puberty	Total
No. examined	56	72	128
No. with xerophthalmia	3	3	6
No. with phrynoderma	13	27	40
No. with one or more signs of vitamin A deficiency	13	28	41

Comment. The results are comparable with those obtained at Assamuk.

4. Results of all Examinations. 1,112 individuals.

Table VI

Children

School, etc.	Xerophthalmia	Phrynoderma	Total with vitamin A deficiency	Total seen	Percentage affected
Ngora E.V. (C.M.S.) ...	5	43	45	145	30·2
„ B.S. (C.M.S.) ...	1	8	9	41	21·9
„ B.S. (girls) ...	1	31	31	115	26·9
„ B.S. (R.C.) ...	6	41	41	128	32·0
„ E.V. (R.C.) ...	5	25	27	74	36·4
Assamuk E.V.	7	26	38	80	47·5
Toroma E.V. (R.C.) ...	2	27	27	68	39·7
„ E.V. (C.M.S.)	3	1	3	59	5·1
Amuria E.V. (C.M.S.)...	1	1	2	59	3·4
Serere E.V.	5	16	18	53	33·9
„ B.S.	2	9	10	30	33·3
Kilitok E.V.	1	14	14	37	37·8
Kamod E.V.	1	14	14	41	34·1
„ B.S.	0	7	7	22	31·8
Adults					
Ngora T.T.S.	2	7	7	48	14·5
County gaol	1	3	3	26	11·5
Adults (Toroma) ...	1	4	4	86	4·6
Totals					
Total children	40	263	286	952	30·0
„ adults	4	14	14	160	8·7
Grand Total	44	277	300	1,112	26·9

Comment. The small proportion for Amuria and Toroma (C.M.S.) schools is attributed to the fact that these pupils are, on the whole, drawn from a wealthy

class, as compared with those attending the neighbouring schools at Assamuk and Toroma (R.C.). See also Appendix.

VI. DISCUSSION

(*a*) *Age*

The results of this investigation support the prevalent belief that vitamin A deficiency shows itself more readily in young than in older children. So far as could be ascertained by careful questioning, there is no suspicion of the younger children being neglected to the advantage of their elders in their own homes, and the results obtained from the boarding-schools, where rations are issued under supervision, confirm this view by giving the same incidence for boarders and non-boarders.

Whether the young growing child requires proportionately more vitamin A than the adult, or whether his storage capacity is relatively less, is not decided. It is clear, however, that three months' vitamin A deficiency causes visible signs in about one half of children under puberty, in about a quarter of adolescents, and in only a very small fraction of adults.

(*b*) *Economic Factors*

In the north-eastern area the social and financial status of the pupils' families has a decided effect : Amuria School educates the sons of chiefs and wealthy peasants, and fees are considerably higher than at Assamuk, where the majority of pupils come from extremely humble surroundings. Further, Amuria is remarkable for the number of cattle owned by the inhabitants ; the children of wealthier individuals would, therefore, have more milk at their disposal than children in most other parts of the district. The solitary pupil found with xerophthalmia at Amuria School explained that he did not drink milk because he had once, some years ago, vomited after drinking a large quantity.

In all parts of Teso the poorness of the 1934 sweet-potato crop may be to some extent blamed for the present prevalence of vitamin A deficiency. In the absence of adequate supplies of milk and green vegetables during the dry season, the use of sun-dried sweet potatoes would probably prevent or mitigate the occurrence of vitamin A deficiency.

(*c*) *Sex*

There is apparently no marked difference in the incidence of this deficiency between the sexes, although females do not eat eggs. The conclusion to be drawn is either that the males do not eat sufficient eggs to increase appreciably their vitamin A reserves, or that they destroy this factor by over-cooking. Apart from eggs, I could find no differences between the diet of the sexes.

(*d*) *Schools*

There is no suggestion that attendance at schools in any way affects the incidence of this deficiency, as boarders eat precisely the same food as day-boys, i.e., the food of the country. On the contrary, schools may be of great

service by supplying cod-liver oil to all their pupils, as is now being done at Assamuk.

(*e*) *Other Deficiency Diseases*

I ascribe the absence of these to the prevalent custom of drinking beer and, possibly, whey of soured milk. The absence of stomatitis, neuritis, etc., and the small incidence of septic diseases seen in this series of cases leads me to believe that such manifestations are complications of other deficiencies, and are not connected with lack of vitamin A. This question is dealt with in a subsequent paper.

(*f*) *Severity*

At present (April, 1935) the native population of Teso is only on the verge of serious vitamin A deficiency. This is shown by the small percentage of adults affected, by the proportionately low incidence of xerophthalmia among children suffering from phrynoderma, and by the absence of cases of keratomalacia.

It is probable that, with the advent of normal rains, the condition will disappear until after the next dry season. Should the crops, however, be affected by a drought or by disease, the deficiency would certainly become more prevalent and severe, and a dearth of beer due to a failed millet crop would possibly cause the appearance of pellagra and other deficiency diseases.

(*g*) *Prevention*

If it can be shown that sun-dried sweet potatoes do not lose their vitamin A content, the prevention of this disease becomes a simple matter. The native must be encouraged to grow this tuber and to store sufficient for the dry season to last him and his children until the next crop is gathered. In the meantime, should the deficiency become more severe, and keratomalacia with consequent blindness be threatened, the schools offer an easy approach to the treatment of a certain proportion of the child population.

An endeavour to increase the milk supply, either by the rearing of more cattle or by persuading parents to give their children the lion's share of the family's supply, is not at present feasible on financial and social grounds. The advantages of drinking fresh, whole milk might, however, be stressed in all schools.

VII. SUMMARY

1. A survey for signs of vitamin A deficiency has been made among the population of Teso, Uganda.

2. One thousand, one hundred and twelve persons have been examined—including 1,000 school pupils—and 300 cases of vitamin A deficiency were found. The incidence among children averages 30 per cent.; that among adults 8·7 per cent.

3. This deficiency is attributed to dietetic, climatic, financial and social factors, which vary in importance in the different areas.

4. The effects of age and sex are discussed.

5. The value of native beer in preventing other deficiency diseases is stressed.

6. An attempt has been made to assess the value of preventive measures.

My thanks are due to the following : The Honourable Dr. W. H. Kauntze, M.B.E., Director of Medical Services, Uganda, for permission to publish this paper ; Mr. M. G. de Courcy-Ireland, Agricultural Officer, Serere, for information about agricultural matters ; the managers of the various schools for their co-operation in this work.

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IX. APPENDIX

The figures for cattle in relation to the number of inhabitants in the four rural districts under review are :—

	Number of cattle	Population (taxpayers)	Index of cattle per head of population
Serere (Osuguro)	6,758	2,012	3·4
Ngora	4,029	1,905	2·1
Kuju (Amuria)	15,287	1,852	8·3
Toroma	11,064	1,953	5·7

(The index is, of course, the number of cattle per taxpayer in each area. To obtain the approximate figure for total population, taxpayers must be multiplied by 4·5.)

Comment:—The relative freedom from vitamin A deficiency of the sons of wealthy parents in Amuria and Toroma is very probably connected with the cattle indices given above.

A FOURTH CASE OF HUMAN INFESTATION WITH *BERTIELLA STUDERI* (CESTODA) IN MAURITIUS

BY

A. R. D. ADAMS, M.D.

(*Received for publication* 26 *July*, 1935)

Three cases of human infestation with *Bertiella studeri* have already been recorded from Mauritius (Blanchard, 1913; Adams and Webb, 1933); the following is the fourth from this island and the tenth actually recorded from man.

A young Hindu boy was observed by his relatives to be passing ‘ worms.’ Specimens were submitted to Dr. Pilot, of Moka Hospital, and the patient was kindly referred to me for investigation. The child was found to be a particularly and unusually robust boy aged 7 years. He appeared in perfect health, both physical and mental, and complained of no symptoms or signs other than the passing of the above-mentioned ‘ worms.’ On clinical examination nothing abnormal could be detected in the child, and the blood picture presented no unusual features. For about a month the patient was watched and passed about every ten days a piece of the strobila of a tape-worm consisting of from ten to twenty gravid segments of what looked remarkably like *Bertiella studeri.* No other helminth infestation was detected in spite of repeated examination, and enquiry elicited the fact that the child had undergone anti-hookworm treatment (carbon tetrachloride) three times during the last year, the last occasion being about three months before the present parasite was first noticed.

In view of the parent's anxiety to rid the patient of his trouble, it was deemed advisable to treat him before he was taken elsewhere for this purpose. An invitation to enter hospital was refused, so, after instructions for preliminary starving for 24 hours, 1·4 c.cm. carbon tetrachloride (0·2 c.cm. for each year of age) was administered in half an ounce of castor oil by the mouth. An hour and a half later a first semi-formed motion was voided and in this was seen the parasite. Two subsequent motions failed to yield more than four young immature Ascaris and a female Enterobius.

Examination of the cestode showed that the worm was complete and intact, with the unfortunate exception of the head which was never found in spite of long search. The strobila was unbroken and appeared to consist of the entire worm from the neck downwards. It measured 33 cm. in length after fixation in formalin between two sheets of glass, and 1·75 cm. at its broadest diameter. The morphology was that of *Bertiella studeri*, when studied in detail, and the terminal segments were gravid and contained the characteristic ova armed with the pyriform apparatus characteristic of the genus.

The patient was under observation for another three months, but no further segments were passed, so that it appears probable either that he has got rid of the head or that the latter was missed at the time of the treatment.

During the original period of observation before treatment, numbers of freshly-passed gravid segments were fed to a young monkey (*Macacus cynomolgus*) which has been at the laboratory since its earliest days. Subsequently segments were fed to this animal after they had been kept moist for varying periods of up to ten days. Four months later there was no indication of successful parasitization of the animal.

SUMMARY

1. A fourth Mauritian case of human infestation with *B. studeri* is recorded.

2. The patient, a Hindu boy aged 7, was in perfect physical and mental health, in spite of his infestation.

3. Treatment with carbon tetrachloride resulted in dislodgement of the worm.

4. Direct feeding of gravid segments, both freshly-passed and after keeping for some days, to a young *M. cynomolgus* failed to cause parasitization of the animal.

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A COMPARATIVE STUDY OF THE MALE AND FEMALE TERMINALIA OF SOME SPECIES OF THE SUBFAMILY TACHININAE

BY

W. S. PATTON

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication* 2 *August*, 1935)

INTRODUCTION

The subfamily Tachininae (family Tachinidae auct.) contains a very large number of muscids which are, in their larval stages, parasites of other insects, and must, therefore, be very ancient members of the family Muscidae. As such, therefore, it is important to make an intensive comparative study of the ♂ and ♀ terminalia of some species in order to see what light they throw, not only on the systematic position and relationships of these flies, but also on those of other higher Diptera, such as *Glossina*, etc. Incidentally, such an intensive study will undoubtedly pave the way to a better understanding of the classification of these important flies. I should like to point out at the outset of this study that I have no special knowledge of the taxonomy of these flies, and therefore I have no particular bias as to the value, or otherwise, of external characters used in classifying them. I shall approach the systematic study of the Tachininae, therefore, strictly from the standpoint of the comparative study of the ♂ and ♀ terminalia, and I shall endeavour to interpret my findings in the light of the very extensive knowledge that I have now gained from the study of the terminalia of many other higher Diptera. I realize that I can only hope to touch the fringe of the subject, since, in the first place, I do not possess the necessary material, having only very casually collected the Tachininae, and secondly the time to go deeply into the subject is now too short. It will remain for others sufficiently enthusiastic to follow up my work. To any such who may be contemplating such a study I should like again to sound a note of warning, but at the same time to say that I am convinced that the terminalia offer the surest guide to such vexed questions as the limits of genera, etc.—questions which as far as I can see cannot be answered by the characters at present used to group these flies.

The comparative study of the ♂ and ♀ terminalia of the higher Diptera if it is to be of any lasting value must be a complete one—by which I mean that the parts must be studied in great detail. This involves a very delicate technique, the dissection and mounting of the various parts without compression, and the orientation of them all in the same way so that one's results are comparable. Orientation of the parts is of vital importance, for the same structure if viewed from another angle (or compressed under a cover-slip)

may appear so different as to lead to entirely erroneous conclusions. Before dissection the worker must study the parts *in toto* and must learn and understand their relationships to each other. It is equally important that the worker should be able to illustrate his specimens, for accurate drawings are of far more value than any number of photographs of the parts. I now know that the ♀ terminalia are of equal, if not of greater, importance than those of the ♂ in understanding the relationships of species ; they often throw important light on some structure present on the ♂ parts which may lead the worker to think that he is dealing with a species generically novel. *Calliphora grahami* Aldrich is a good example of this fact. Lastly, it is essential to have at one's disposal a large and varied collection, so that not only many genera can be studied but also many species of each genus. One never knows when a connecting link, or a species which at once throws light on the relationships of others, may not turn up. I have lately had a most striking example of this, which I shall in due course record.

I should like to take this opportunity of expressing my grateful thanks to Mr. Colbran J. Wainwright, F.R.E.S., the well-known authority on these flies, for the specimens which he has so generously given me ; without his help this (and any subsequent studies) would have been quite impossible. I also wish to thank Dr. Baranoff for the gift of specimens of his species *Echinomyia pandelléi* and specimens of *E. praeceps aestivalis* Baranoff. Whether or not I shall be able to continue these studies will depend entirely on the generosity of my readers. I will give a list of some genera, species of which I should like to study. I need hardly say that I shall make the best possible use of any such material. It is necessary to point out that in the case of the present study I have only been able (except in the case of *E. fera* L.) to study single specimens, and therefore cannot express any opinion as to individual variation in the structure of the parts. In the present paper I am illustrating in detail the ♂ and ♀ terminalia of the following species (I have not had an opportunity of examining the ♀ terminalia of *E. pandelléi* Baranoff) :—

Echinomyia fera L.
Echinomyia (*Eudoromyia*) *magnicornis* Zett.
Echinomyia (*Fabriciella*) *ferox* Panz.
Echinomyia (*Fabriciella*) *pandelléi* Baranoff
Echinomyia grossa L.
Echinomyia (*Servillia*) *ursina* Meigen
Echinomyia (*Servillia*) *lurida* F.

As the segmentation of the abdomen in these flies is similar to that of *Musca*, etc., which I have fully described in earlier papers, I do not propose to describe it again. In the case of these seven species tergum 6 appears to be wanting and sternum 6 is usually a large plate incomplete on the right. The other sternites appear to be normal, and the terga usually come close up to their lateral edges. Tergum 5 in both sexes offers some good taxonomical

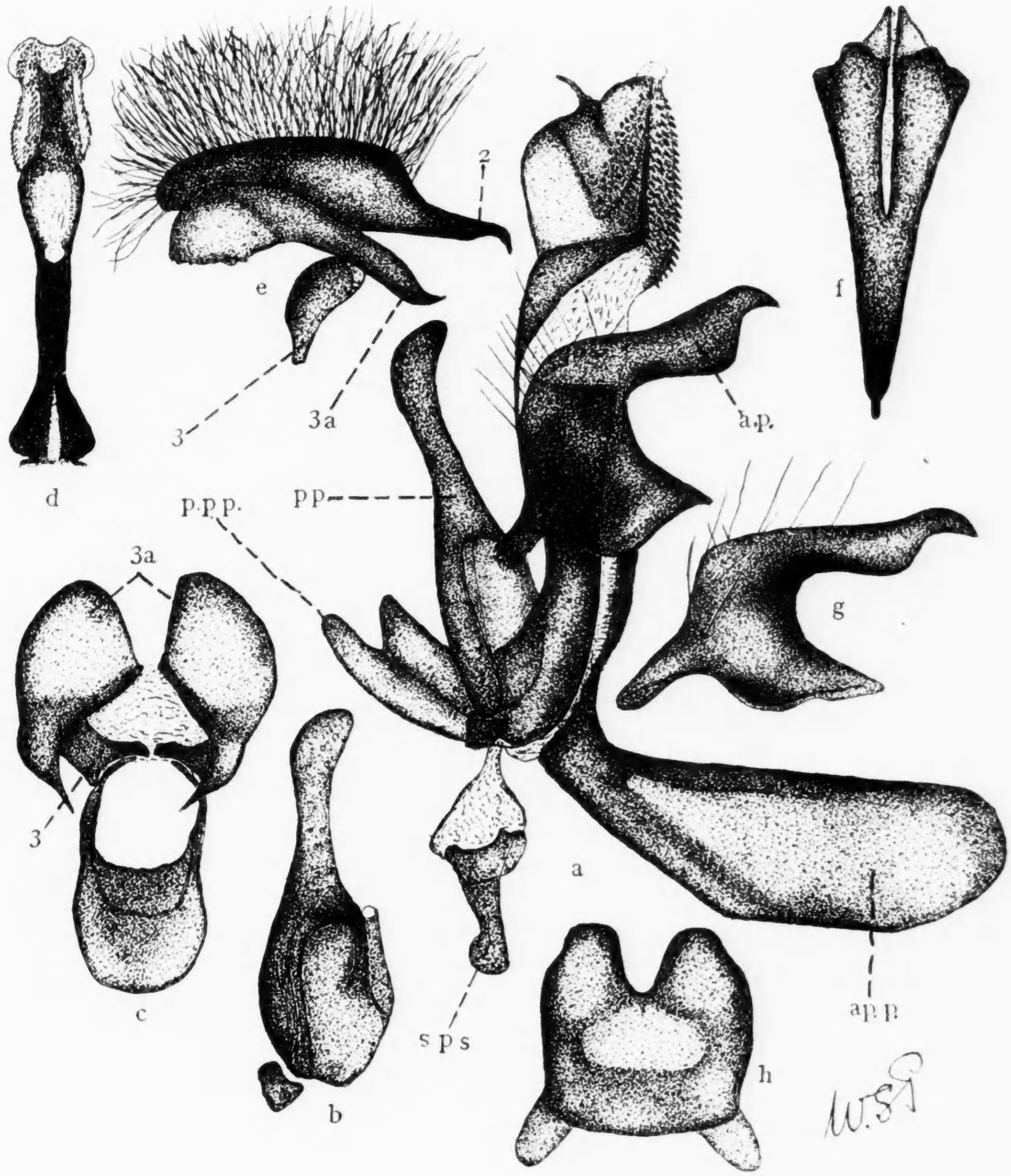

FIG. 1. Terminalia of ♂ *E. fera*. *a.*—Phallosome, paramere (*a.p.*, anterior part; *p.p.*, posterior part in side view; *ap.p.*, apodeme of phallosome; *p.p.p.*, posterior process of phallosome; *s.p.s.*, sperm pump sclerite); *b.*—Left anterior paramere; *c.*—Ninth coxites showing method of articulation with ninth tergo-sternum (3, proximal, 3*a*, distal segment of ninth coxite); *d.*—Dorsal view of end of phallosome; *e.*—Anal cerci and one ninth coxite in side view (lettering as in *c*); *f.*—Ventral view of fused anal cerci; *g.*—Left anterior part of paramere in side view; *h.*—Fifth sternum.

characters of subsidiary use. As the various parts of the terminalia are very fully illustrated in each case, I intend to give only a short description of those of *fera* as a type of the genus, and shall only refer to important differences in the parts in the case of the other species. All the drawings are to scale—i.e., the phallosome, parameres, ninth coxite, etc., of each species is drawn to the same

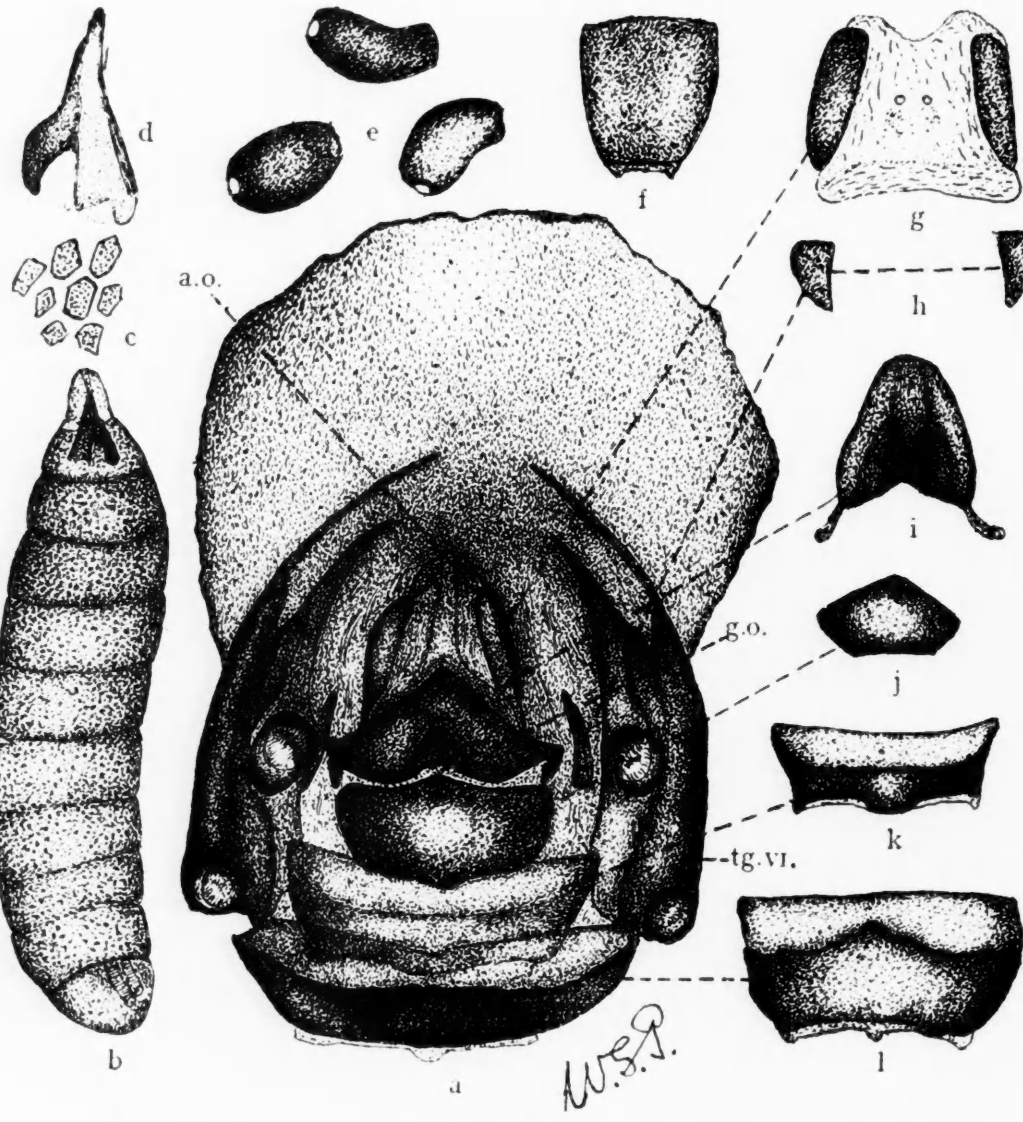

FIG. 2. *a.*—Ventral view of terminalia of ♀ *E. fera* showing extended larvipositor (*a.o.*, anal opening; *g.o.*, genital opening; *t.g. vi.*—Sixth tergum); *b.*—First stage larva; *c.*—Cuticular markings of same; *d.*—Cephalopharyngeal skeleton of same; *e.*—Spermathecae; *f.*—Fifth sternum; *g.*—Anal cerci; *h.*—Seventh tergum; *i.*—Tenth sternum; *j.*—Ninth sternum; *k.*—Seventh sternum; *l.*—Sixth sternum.

scale; and with the exception of the drawing of the entire terminalia of the ♀ *grossa* this applies to the ♀ terminalia as well. In the case of the ♀ terminalia, as before I have illustrated the entire larvipositor as seen from the ventral side, and have then dissected off the sclerites, mounted them more or less flat, and drawn them at the sides. It will be obvious to any one who has studied such

whole mounts that the individual sclerites appear foreshortened and in many respects quite unlike the more accurate drawings at the sides, which are on a smaller scale.

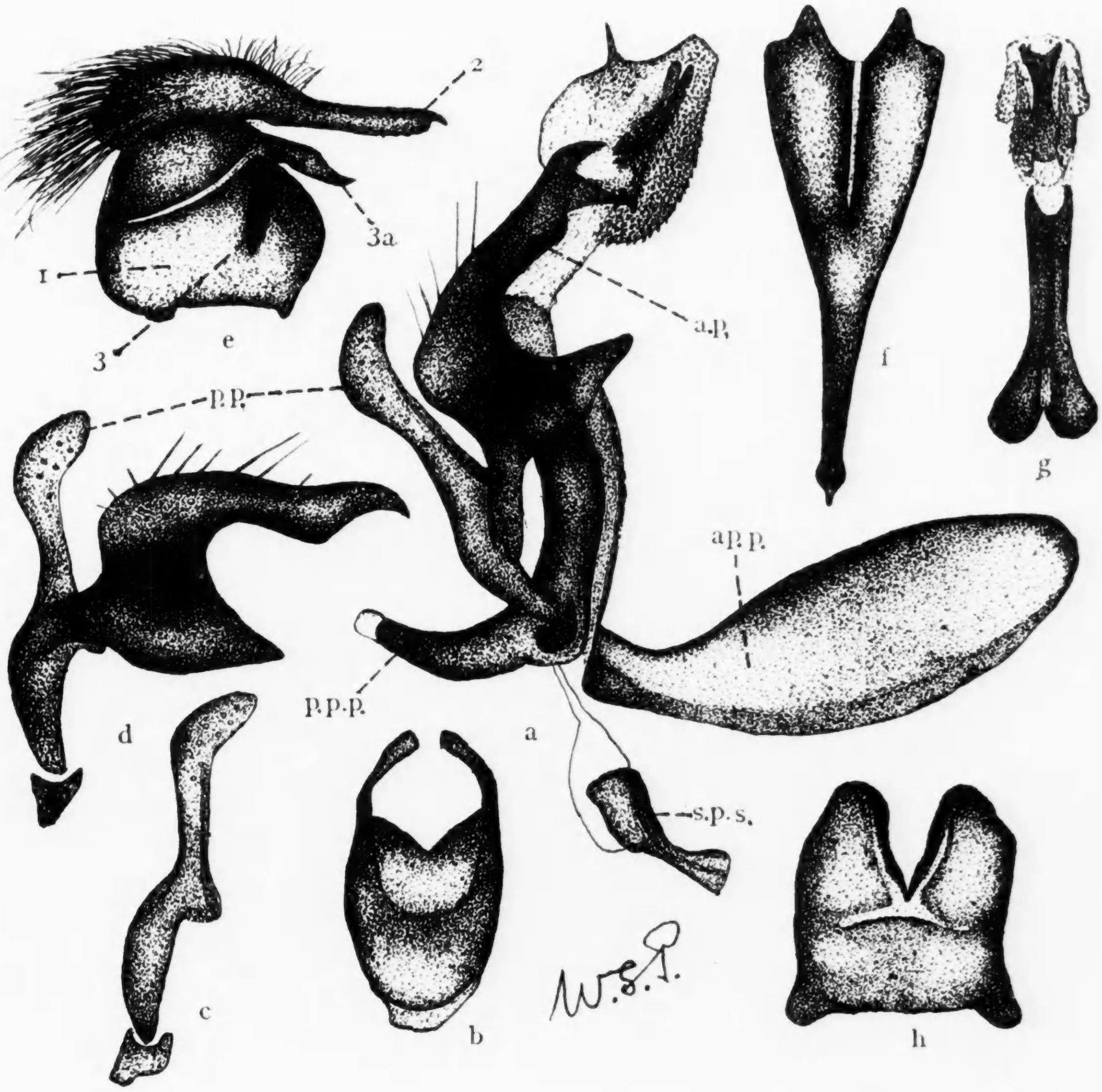

FIG. 3. Terminalia of ♂ *E. magnicornis*. *a*.—Phallosome and paramere in side view (lettering as in fig. 1, *a*); *b*.—Ninth tergo-sternum; *c*.—Right posterior paramere; *d*.—Right paramere (two parts) in side view; *e*.—Anal cerci, one ninth coxite and tenth tergum in side view (1, tenth tergum; other lettering as in fig. 1, *e*); *f*.—Ventral view of fused anal cerci; *g*.—Dorsal view of end of phallosome; *h*.—Fifth sternum.

SHORT DESCRIPTIONS OF THE MALE AND FEMALE TERMINALIA OF *ECHINOMYIA FERA* L.

Echinomyia fera L. SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. Fig. 1. SCLERITES. Tergum 5 as in fig. 1, *h*. The ninth tergo-sternum (fig. 1, *c*) is a massive plate, the posterior processes very wide, overlapping and closely bound down with the sides of the posterior process of the

phallosome ; it varies in structure in the different species, as will be noted in the drawings. APPENDAGES. *Ninth Coxite. Distal Segment.* Ventral view. Fig. 1, *c*. Short, turned inwards and ending in a sharp point ; note the position of the distal segments in relation to the fused anal cerci. Lateral view as in fig. 1, *e*, which shows the backward prolongation fusing with the sides of tergum 10. *Proximal Segment.* Lateral view. Fig. 1, *e*. A broad plate

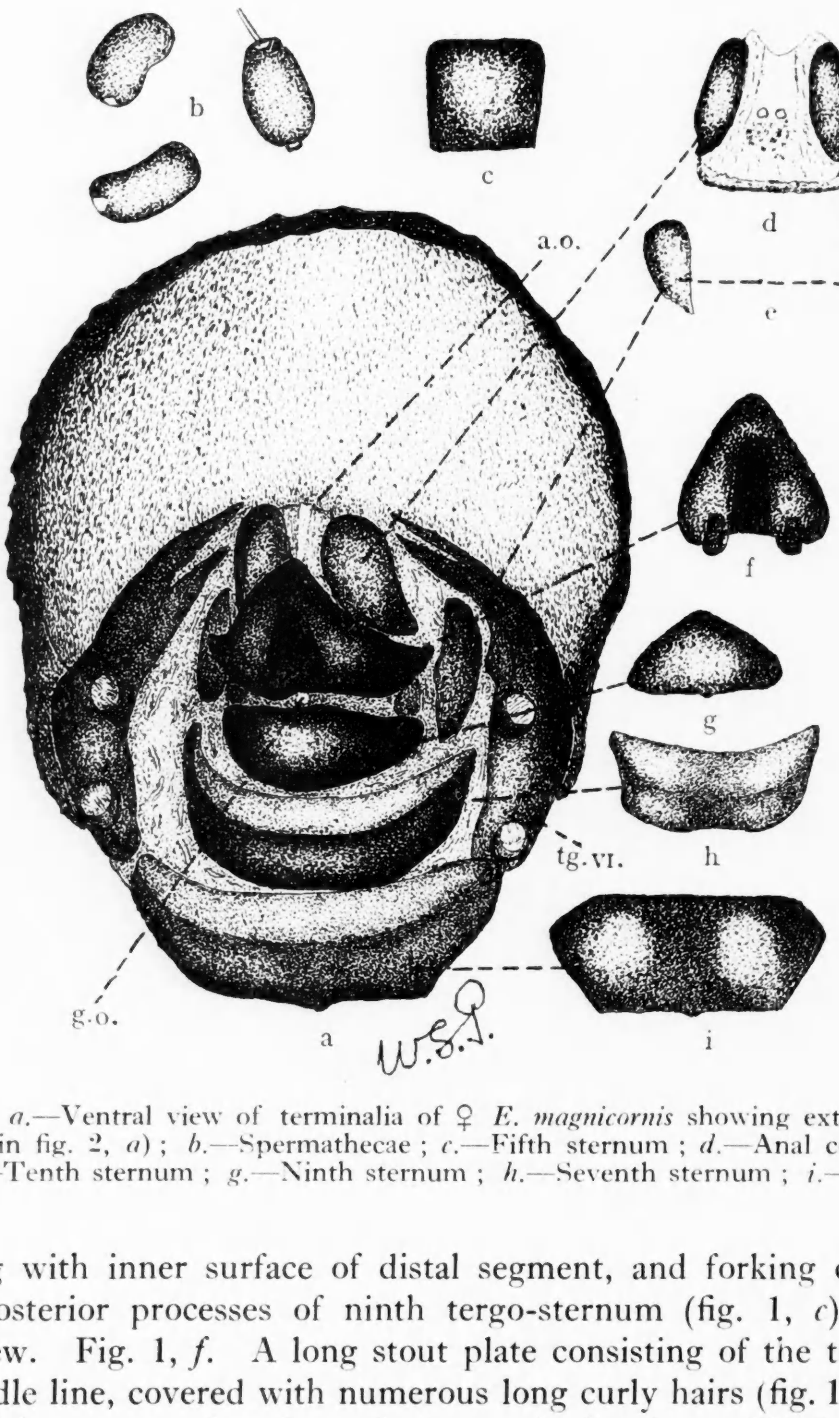

FIG. 4. *a*.—Ventral view of terminalia of ♀ *E. magnicornis* showing extended larvipositor (lettering as in fig. 2, *a*) ; *b*.—Spermathecae ; *c*.—Fifth sternum ; *d*.—Anal cerci ; *e*.—Seventh tergum ; *f*.—Tenth sternum ; *g*.—Ninth sternum ; *h*.—Seventh sternum ; *i*.—Sixth sternum.

articulating with inner surface of distal segment, and forking closely applied to wide posterior processes of ninth tergo-sternum (fig. 1, *c*). *Anal Cerci.* Ventral view. Fig. 1, *f*. A long stout plate consisting of the two cerci fused in the middle line, covered with numerous long curly hairs (fig. 1, *e*), narrowing to end and bent upwards. Lateral view as in fig. 1, *e*. *Phallosome.* Lateral view. Fig. 1, *a*. Long, narrow, the proximal part forming a long chitinous

tube ; distal part formed mainly by fused struts which bend upwards and are continued medially by a characteristic plate (its extent and chitinization varies in the different species) ; two chitinous spined flaps on each side, as shown in fig. 1, *a*, *d*. *Posterior Process of Phallosome*. Fig. 1, *a*. Short, wide and forked at end, firmly attached to posterior processes of ninth tergo-sternum. *Sperm Pump Sclerite*. As in fig. 1, *a*. *Apodeme of Phallosome*. Fig 1, *a*. A rather short wide plate as seen in side view. *Parameres*. Lateral view. *Anterior Part*. Fig. 1, *a*, *g*. A rather long bent plate, ending in a sharp point with a row of hairs on outer ventral border. *Posterior Part*. Fig. 1, *a*, *b*. A rounded upstanding plate.

Salient Diagnostic Characters of Female Terminalia. Larvipositor. Segments 6, 7, 9 and 10. Fig. 2. The entire ♀ terminalia are illustrated in fig. 2, which shows the extensive membranous area between the edges of tergum 5 and the two parts of tergum 6. The sclerites forming the larvipositor are illustrated at the sides (on a smaller scale) from specimens dissected off and mounted flat to show their exact structure. It should be noted that the sclerites in the drawing of the whole mount appear often structurally different from those drawn from separated specimens. The important facts connected with the terminalia are that they represent a good example of a simple larvipositor, for, except for terga 9 and 10 (which have become reduced), all the sclerites characteristic of a short ovipositor are here present. Tergum 6 (fig. 2, *a*) consists of two separate plates joined by membrane, and this may be characteristic of the genus ; each bears spiracle 6 and 7. The genital opening lies between sternum 9 and 10, the vagina being a narrow tube opening in such a way that the little larva when being extruded passes along the groove on the ventral surface of tergum 10 ; the groove (which varies in depth and length in the different species) probably allows of some precision in the deposition of the larva. Tergum 7 consists of two small, somewhat triangular plates ; they are better developed in some species than in others. Note the position of the anal opening and the anal cerci. The three spermathecae are illustrated in fig. 2, *e*.

The first stage larva is illustrated in fig. 2, *b*. Like all the similar larvae of this genus so far studied, it has a characteristic shagreened appearance, due to the surface-markings which consist of somewhat polygonal areas with minute mamillae. The cephalopharyngeal skeleton (fig. 2, *d*) is not strongly developed nor particularly strengthened for boring ; there are minute recurved spines on the sides close to the oral opening. Compare this cephalopharyngeal skeleton with that of the larva of *ferox* (fig. 10, *e*), the fused oral hooks of which form a long, pointed, curved, claw-like structure admirably adapted for piercing ; the end of the cephalopharyngeal skeleton of the larva of *grossa* (fig. 13, *b*) on the other hand is more or less blunt. The ♀♀ of *fera* dissected contained many hundreds of these small larvae, suggesting that they are deposited either on foliage in the immediate neighbourhood of the caterpillar host or where

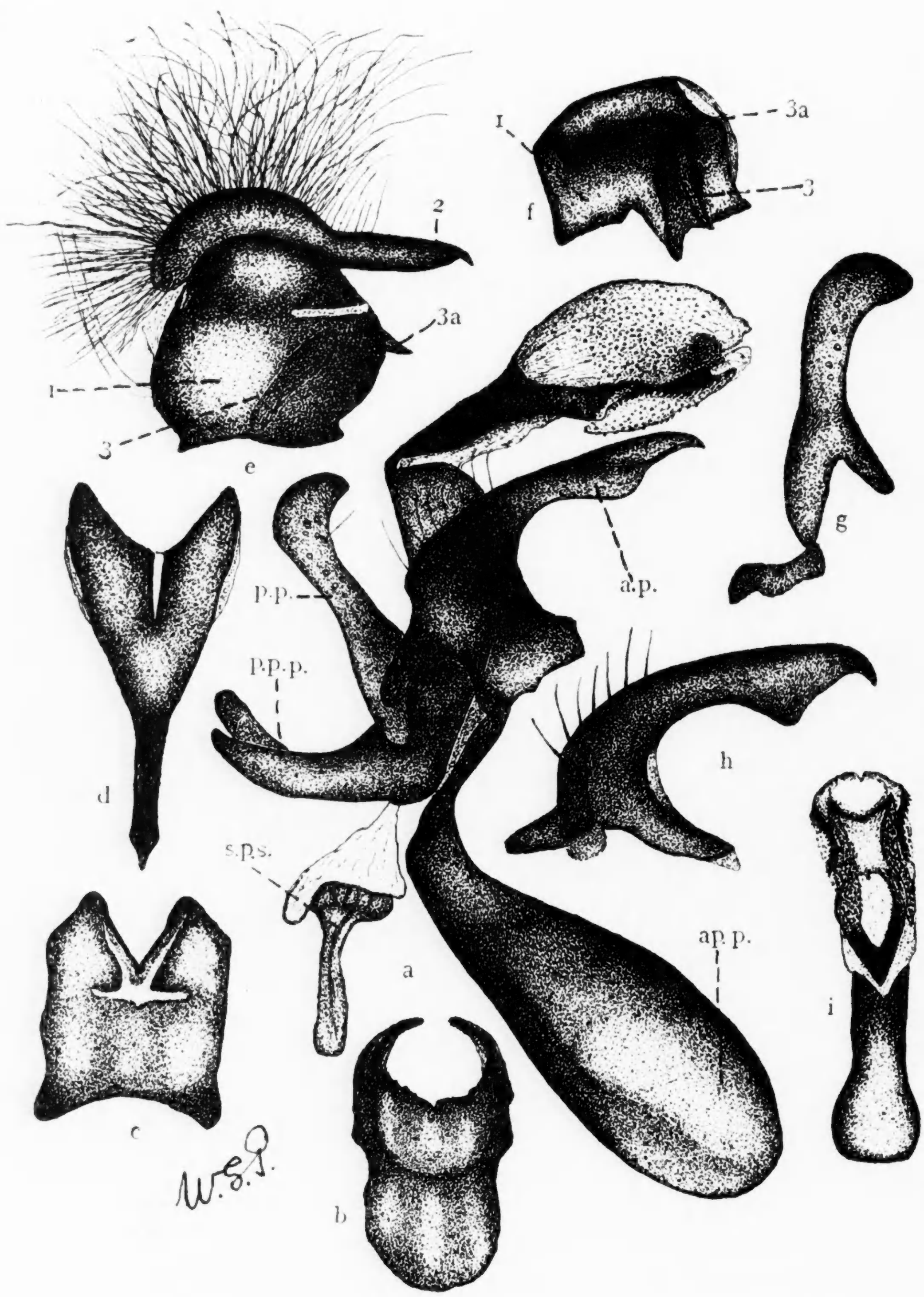

FIG. 5. Terminalia of ♂ *E. ursina*. *a.*—Phallosome and paramere in side view (lettering as in fig. 1, *a*); *b.*—Ninth tergo-sternum; *c.*—Fifth sternum; *d.*—Ventral view of fused anal cerci; *e.*—Anal cerci, one ninth coxite and tenth tergum (lettering as in figs. 1, *e*; 3, *e*); *f.*—Inner view of part of tenth tergum showing two parts of ninth coxite (lettering as in figs. 1, *e*; 3, *e*); *g.*—Right posterior paramere; *h.*—Right anterior paramere; *i.*—Dorsal view of end of phallosome.

it is likely to be feeding ; I have not had time to search the literature to see if it is known exactly how and where the larvae are deposited ; they may be deposited directly on the host. *E. fera* has been bred from several lepidopterous larvae.

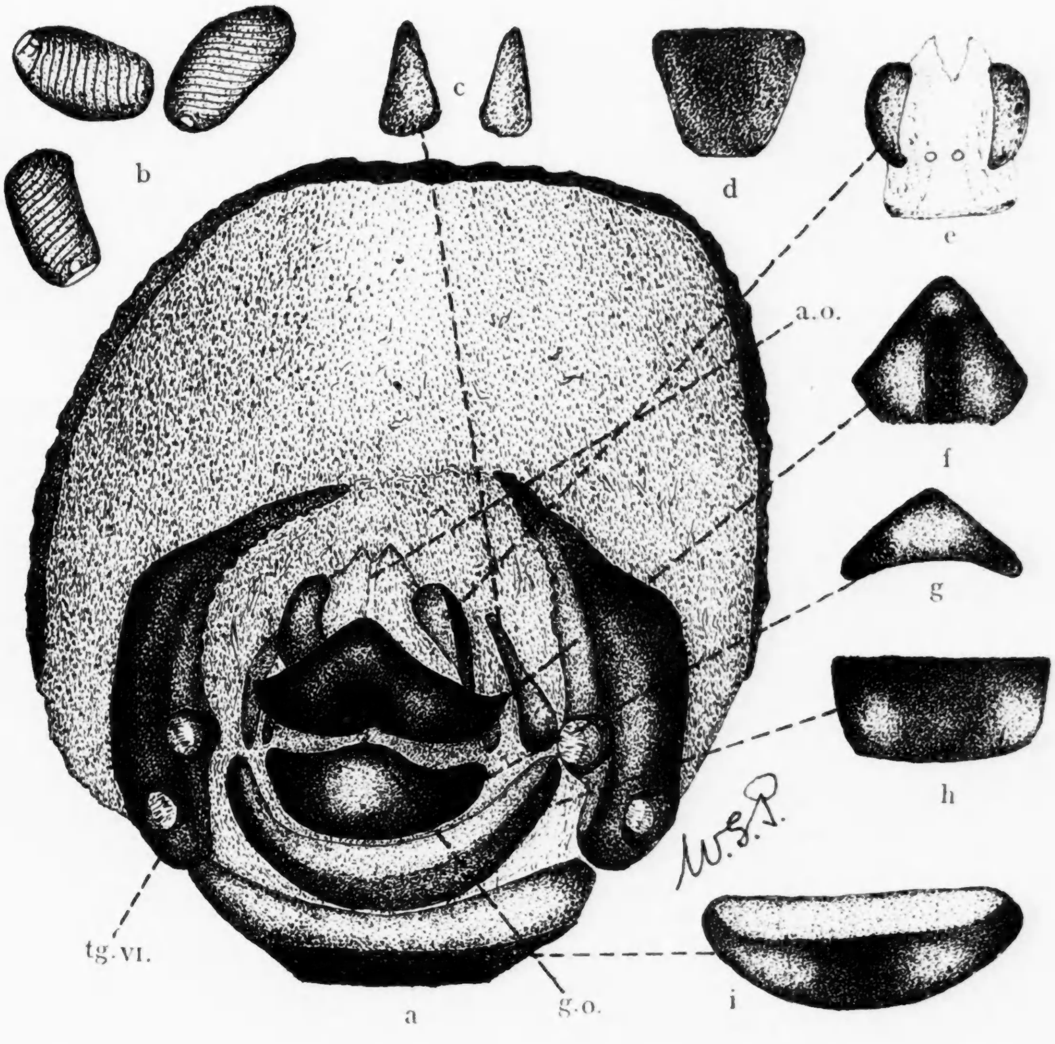

FIG. 6. *a.*—Ventral view of terminalia of ♀ *E. ursina* showing extended larvipositor (lettering as in fig. 2, *a*) ; *b.*—Spermathecae ; *c.*—Seventh tergum ; *d.*—Fifth sternum ; *e.*—Anal cerci ; *f.*—Tenth sternum ; *g.*—Ninth sternum ; *h.*—Seventh sternum ; *i.*—Sixth sternum.

Echinomyia (*Eudoromyia*) *magnicornis* Zett. The ♂ and ♀ terminalia of this species are illustrated in figs. 3 ; 4. A comparison of these illustrations with those of the terminalia of *fera* (figs. 1 ; 2) will leave no doubt that these two species are very closely related, the differences in the parts being very slight. The ♂ anal cerci are distinctly longer, the bent end being shorter ; the distal segment of the ninth coxite of *magnicornis* is shorter than that of *fera* ; the phallosome and parameres are identical. The ♀ terminalia too are very similar, there being only minor differences in the structure of the sclerites.

The first stage larva also is very like that of *fera*. This species was introduced into the United States of America to control the gypsy-moth.

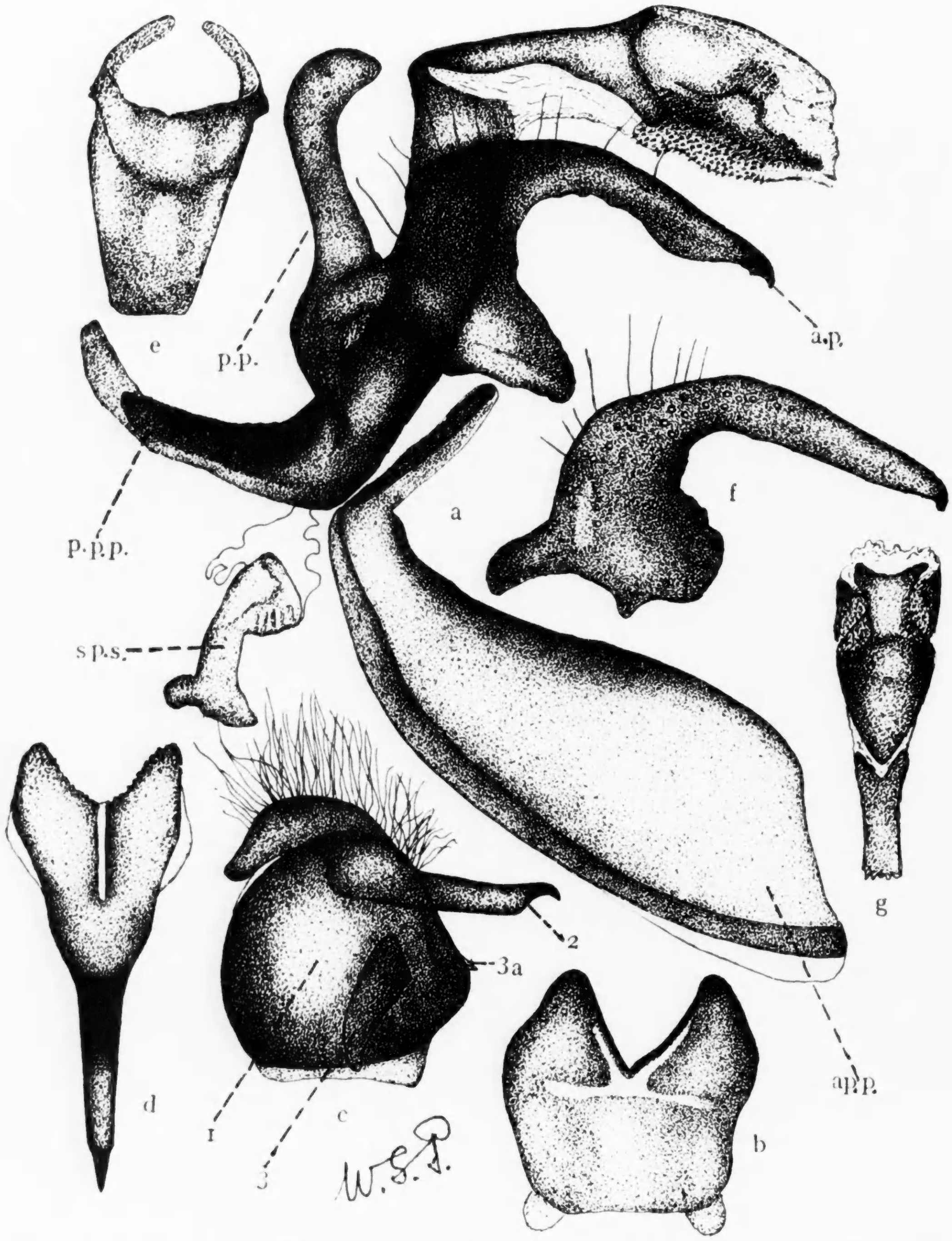

FIG. 7. Terminalia of ♂ *E. lurida*. *a*.—Phallosome and paramere in side view (lettering as in fig. 1, *a*) ; *b*.—Fifth sternum ; *c*.—Anal cerci, one ninth coxite and tenth tergum (lettering as in figs. 1, *e* ; 3, *e*) ; *d*.—Ventral view fused anal cerci ; *e*.—Ninth tergo-sternum ; *f*.—Right anterior paramere ; *g*.—Dorsal view of end of phallosome.

Echinomyia (*Servillia*) *ursina* Meigen. The ♂ and ♀ terminalia of this species are illustrated in figs. 5 ; 6. Here again a comparison with those of *fera* and *magnicornis* demonstrates the fact that this species is also very closely related to them.

Echinomyia (*Servillia*) *lurida* F. The ♂ and ♀ terminalia of this species are illustrated in figs. 7 ; 8 ; like those of its near ally *ursina* they are strikingly like those of *fera* and *magnicornis*. Baranoff recognises two sub-species, *lurida lurida* (F.) Meigen and *lurida leucocoma* Mg.; I have not had an opportunity of studying the terminalia of either.

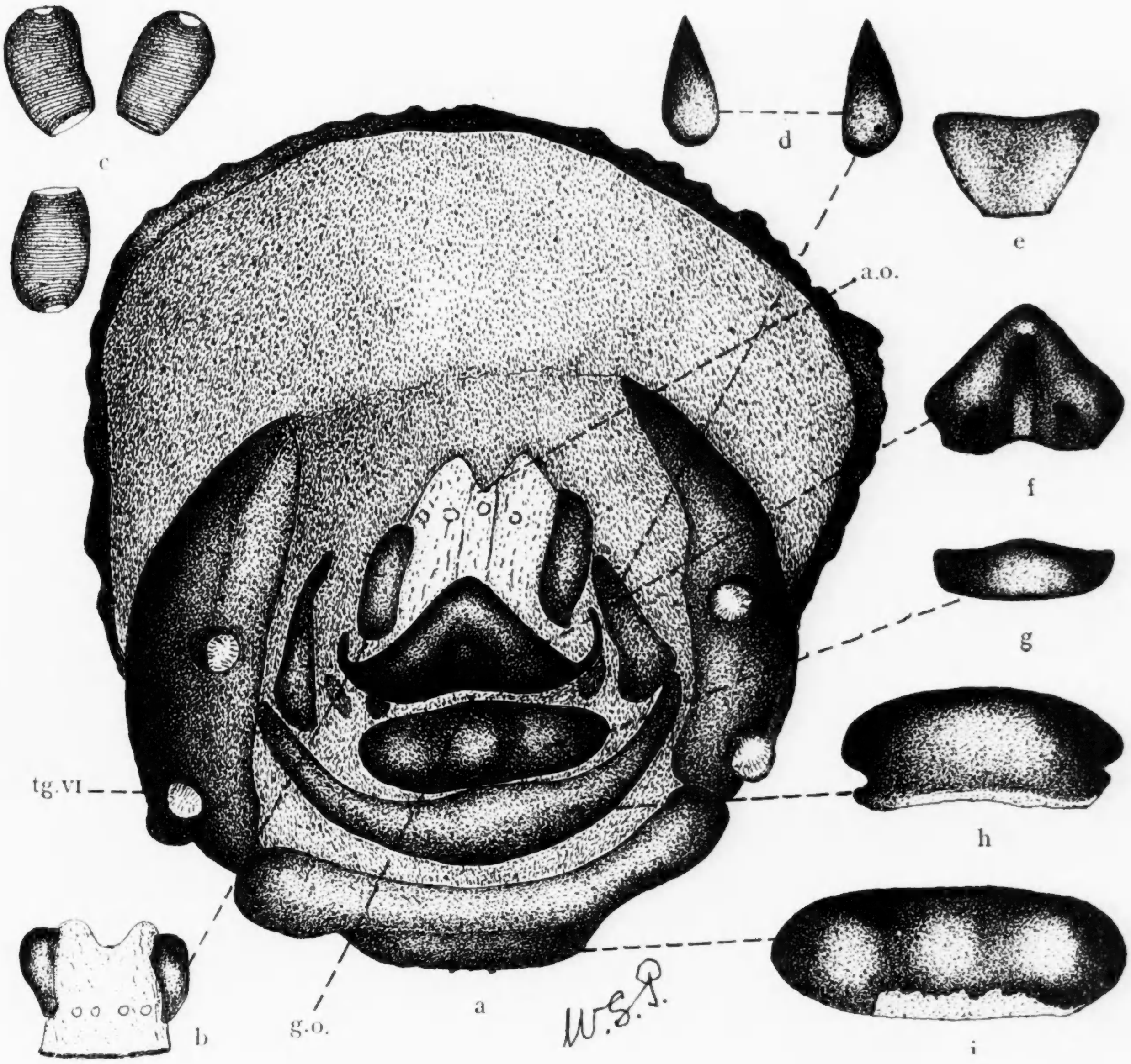

FIG. 8. *a.*—Ventral view of terminalia of ♀ *E. lurida* showing extended larvipositor (lettering as in fig. 2, *a*) ; *b.*—Anal cerci ; *c.*—Spermathecae ; *d.*—Seventh tergum ; *e.*—Fifth sternum ; *f.*—Tenth sternum ; *g.*—Ninth sternum ; *h.*—Seventh sternum ; *i.*—Sixth sternum.

Echinomyia (*Fabriciella*) *ferox* Panz. The ♂ and ♀ terminalia of *ferox* are illustrated in figs. 9 ; 10. In this species it will be noted that the phallosome is much longer and stouter than that of either of the preceding species ; otherwise it is structurally similar. The anterior paramere is more acutely bent. The fused anal cerci provide a striking diagnostic character, which consists

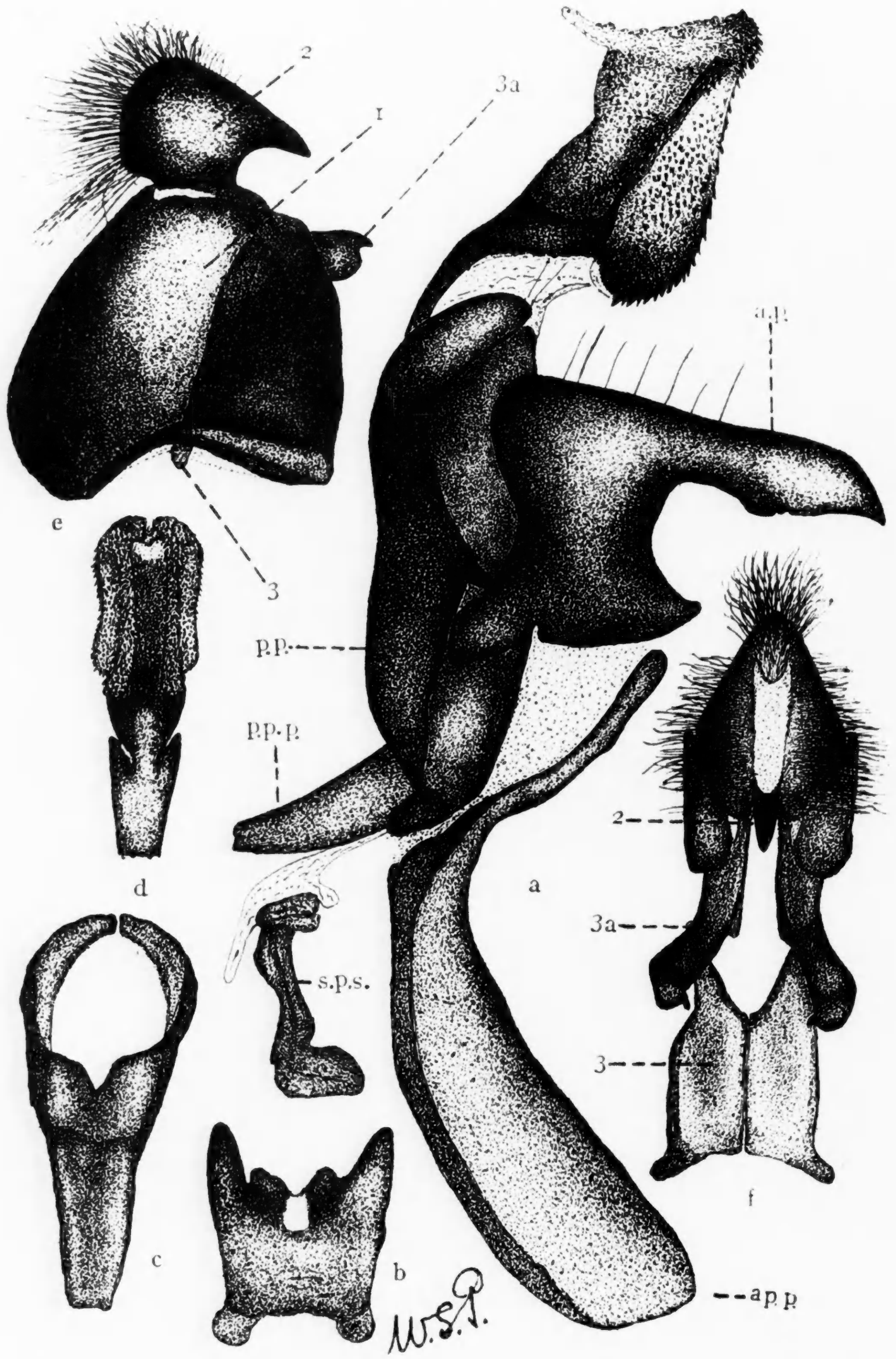

FIG. 9. Terminalia of ♂ *E. ferox*. *a.*—Phallosome and paramere in side view (lettering as in fig. 1, *a*) ; *b.*—Fifth sternum ; *c.*—Ninth tergo-sternum ; *d.*—Dorsal view of end of phallosome ; *e.*—Anal cerci, one ninth coxite and tenth tergum in side view (lettering as in figs. 1, *e* ; 3, *e*) ; *f.*—Ventral view of anal cerci, ninth coxites showing relation to each other (note fused proximal segments, 3).

of a small rounded plate (in side view fig. 9, *e*) ending in a blunt point ; they are very suggestive of those of *Hypoderma bovis* (see *supra*, p. 305). The distal segment of the ninth coxite is longer and broader, ending in a characteristic

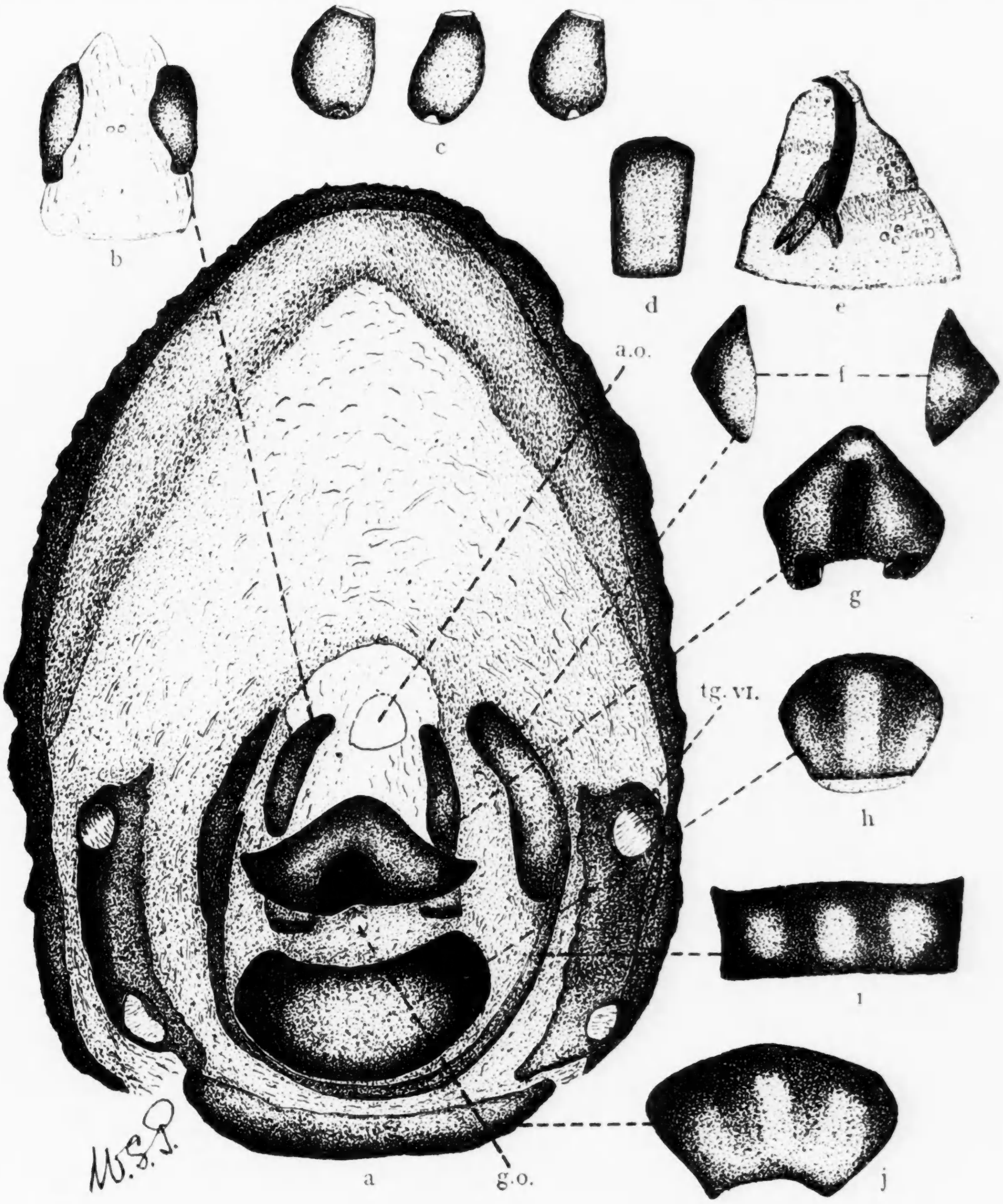

FIG. 10. *a.*—Ventral view of ♀ terminalia of *E. ferox* showing extended larvipositor (lettering as in fig. 1, *a*) ; *b.*—Anal cerci ; *c.*—Spermathecae ; *d.*—Fifth sternum ; *e.*—End of first stage larva showing cephalopharyngeal skeleton ; *f.*—Seventh tergum ; *g.*—Tenth sternum ; *h.*—Ninth sternum ; *i.*—Seventh sternum ; *j.*—Sixth sternum.

inwardly directed lateral clasper. As a result of the greater depth of tergum 10, the proximal segment is long and fused medially with its fellow (fig. 9, *f*). What is the explanation of the small fused anal cerci ? I believe that they represent

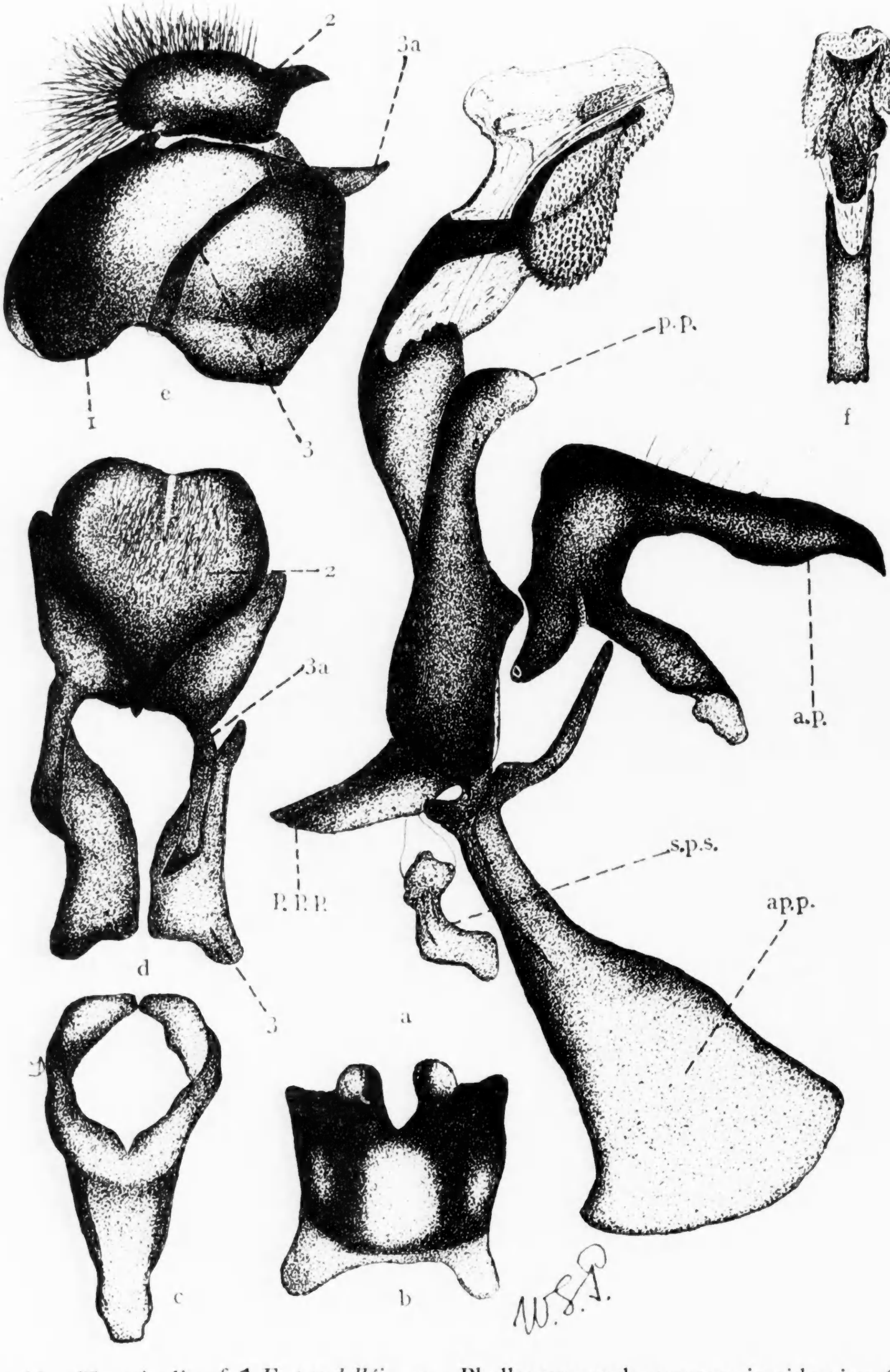

FIG. 11. Terminalia of ♂ *E. pandelléi*. *a.*—Phallosome and paramere in side view (lettering as in fig. 1, *a*) ; *b.*—Fifth sternum ; *c.*—Ninth tergo-sternum ; *d.*—Ventral view of anal cerci and ninth coxites showing relations to each other ; *e.*—Anal cerci, one ninth coxite and tenth segment in side view (lettering as in figs. 1, *e* ; 3, *e*) ; *f.*—Dorsal view of end of phallosome.

the retention of the ancestral type in which they were small, as in *Hypoderma*, *Gasterophilus*, etc.

The ♀ terminalia (fig. 10) are structurally very similar to those of the preceding species. The membranous area between tergum 5 and 6 is narrower but longer than in the preceding species. Tergum 7 is much better developed.

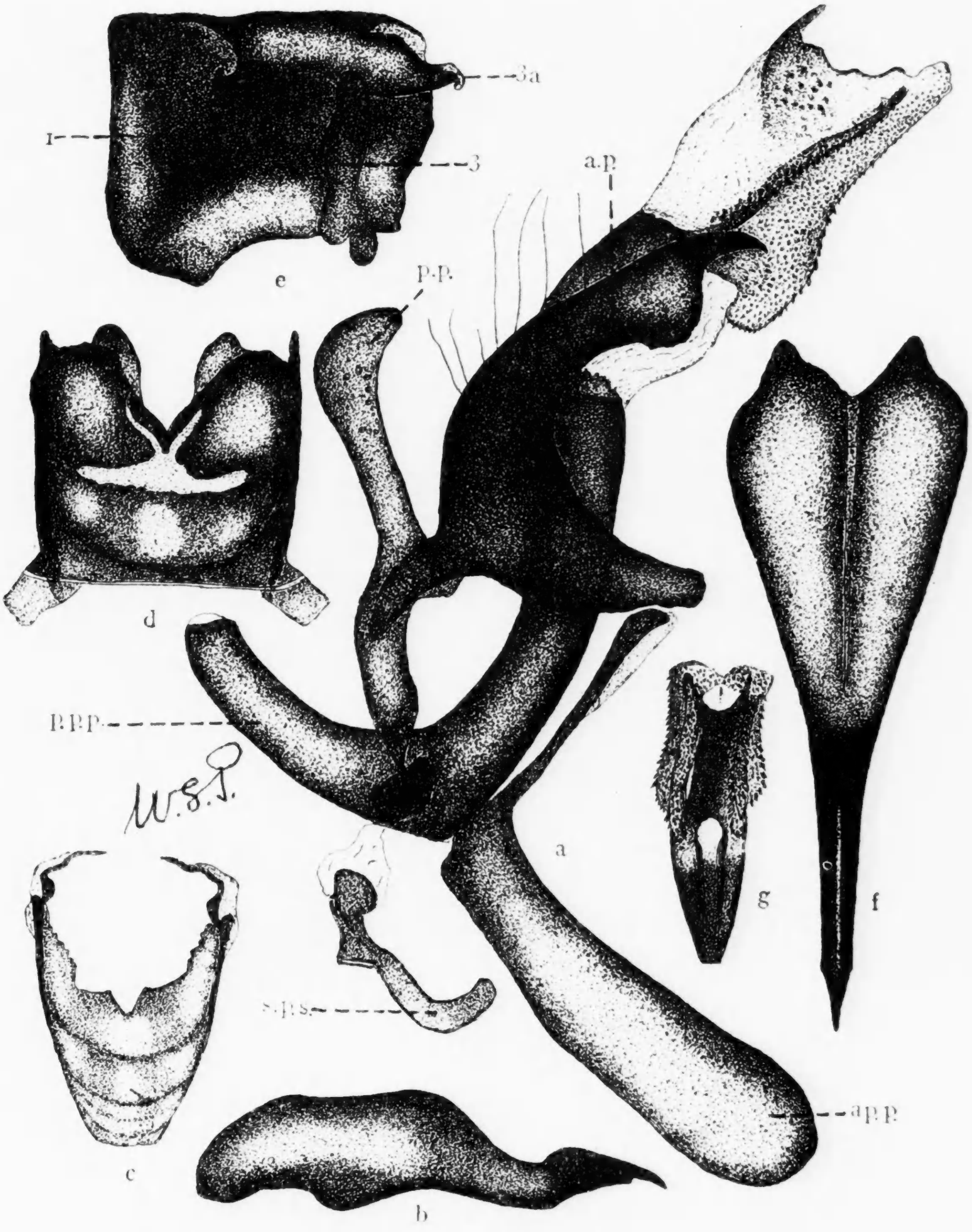

FIG. 12. Terminalia of ♂ *E. grossa*. *a.*—Phallosome and paramere in side view (lettering as in fig. 1, *a*); *b.*—Anal cerci in side view; *c.*—Ninth tergo-sternum; *d.*—Fifth sternum; *e.*—Inner view of part of tenth tergum showing two parts of ninth coxite (lettering as in figs. 1, *e*; 3, *e*); *f.*—Ventral view of fused anal cerci; *g.*—Dorsal view of end of phallosome.

The cephalopharyngeal skeleton of the first stage larva is illustrated in fig. 10, *e*.

Echinomyia (*Fabriciella*) *pandelléi* Baranoff. The ♂ terminalia of this species is illustrated in fig. 11. Baranoff came to the conclusion that this species (which Pandellé regarded as a variety of *ferox*) is distinct from *ferox*.

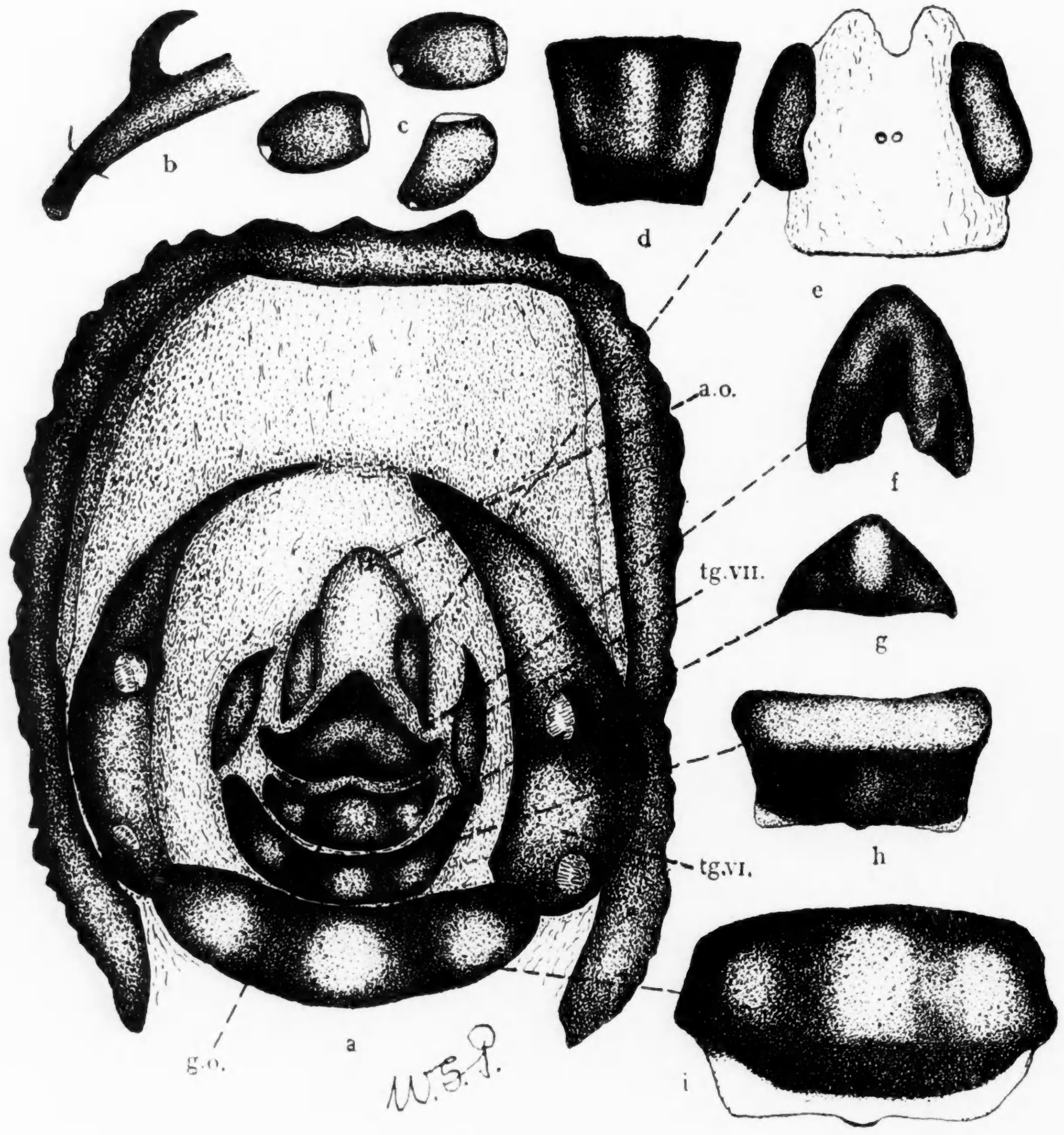

FIG. 13. *a.*—Ventral view of ♂ terminalia of *E. grossa* (lettering as in fig. 2, *a*) ; *b.*—Cephalopharyngeal skeleton of first stage larva ; *c.*—Spermathecae ; *d.*—Fifth sternum ; *e.*—Anal cerci ; *f.*—Tenth sternum ; *g.*—Ninth sternum ; *h.*—Seventh sternum ; *i.*—Sixth sternum.

The specimens which I have studied confirm this view. The important ♂ terminalic differences are brought out in the illustrations. The most striking difference is to be noted in the structure of the distal segment of the ninth coxite ; in *ferox* it is wider and the inturned end terminates in a finger-like

process ; in *pandelléi*, on the other hand, it is narrower and the inturned end is pointed and has a well-marked hook on the inner side (fig. 11, *d*). The anal cerci (fig. 9, *e*) of *ferox* are short and beak-like and bend to the point ; in *pandelléi* (fig. 11, *e*) they are slightly longer and the end is straighter. The phallosome of *pandelléi* is narrower and the posterior part of the paramere is straighter. Sternum 5 is slightly different in the two species (cf. figs. 9, *b* ; 11, *b*). I have not studied the ♀ terminalia.

Echinomyia grossa L. The ♂ terminalia of *grossa* are illustrated in fig. 12 ; it will be noted that they are strikingly like those of the preceding species. The fused anal cerci (fig. 12, *f*) are very long and characteristically shaped, especially when viewed in profile (fig. 12, *b*). The distal segment (fig. 12, *e*) of the ninth coxite is wider, narrowing to the bifid inturned end. The tube-like portion of the phallosome is very narrow, and the posterior paramere is rather short. The ♀ terminalia are illustrated in fig. 13, and it will be noted that they are strikingly like those of the preceding species, there being only slight differences in the shape of the individual sclerites. The cephalopharyngeal skeleton of the first stage larva is illustrated in fig. 13, *b*.

NOTES ON THE SYSTEMATIC POSITION AND RELATIONSHIPS OF THE SPECIES. Before briefly considering the systematic position and relationships of these seven species it is necessary to point out some important structural characters of the terminalia common to them all. In the first place it should be noted that the distal segment of the ninth coxite is in the position of a lateral clasper, and that its structure is correlated with that of the short larvipositor. It varies in structure in the different species, and may either end in a single point directed inwards (it may have a sharp hook at the end on the inner side, as in *pandelléi*) or it may be wider and bifid at the end. The proximal segment is usually fused with its fellow in the middle line, and forking is firmly bound down to the wide posterior processes of the ninth tergo-sternum. When the fused anal cerci are drawn back to grasp the end of the abdomen, the genital fossa or atrium is opened to allow of adjustment with the end of the larvipositor, and the distal segments of the ninth coxites are drawn in towards the middle line as lateral claspers. As I have not seen any of the species taken *in cop.*, I do not know exactly where the ends of the distal segments of the ninth coxites grasp the larvipositor.

The phallosome and parameres are identical in structure in all the seven species, demonstrating their close relationships to each other. In all, the proximal part of the phallosome is a *long chitinous tube* continuous with the short, wide, bifid posterior process. The distal part too is identical in all, and consists of the fused struts which expand and, bending upwards, become continuous with a central chitinous portion (looking like a rod in side view) with a pair of spined flaps on each side.

The ninth tergo-sternum is a massive plate varying in length, its posterior processes characteristically expanded so that they overlap the posterior process

of the phallosome. The two parts of the paramere are also very similar in the seven species.

The structure of the anal cerci are of interest. In all, they have fused in the middle line—a very common change in the higher Diptera from the ancestral small separate plates, as in *Gasterophilus* (see *supra*, p. 310). In five of the species the fused cerci are elongate, and are commonly bent at the pointed end, forming an admirable clasper of the end of the abdomen. In *ferox* and *pandelléi* the fused cerci are relatively small, and are very suggestive of those of *H. bovis* (see *supra*, p. 305). It is difficult to give an adequate explanation of why in two species, which are obviously related to the other five, the anal cerci should have remained in their more primitive condition, but it may perhaps be due to the difference in the shape of the latero-ventral edges of tergum 5 in the ♀ (at least in *ferox*) and to the increase in the length of the phallosome.

Turning to the ♀ terminalia it will be clear from the drawings that in all five they are built on the same plan and are very similar, forming a short larvipositor. In all, tergum 6 consists of only two plates which are widely separated, being joined posteriorly by membrane. The latero-ventral edges of tergum 5 are widely separated. Tergum 7 consists of two short triangular-shaped plates, and tergum 9 and 10 seem to have disappeared. Sternum 9 and 10 are well adapted for the deposition of the small larvae, the concavity on the ninth and the groove on the tenth allowing of precision in its deposition. As noted above, I do not know how or where these small larvae are deposited.

The structure of the ♂ and ♀ terminalia of the species demonstrates beyond any doubt their close relationships to each other. This was recognized by Baranoff (1929), who has studied the ♂ terminalia. It is evident from his work that *Mikia magnifica* Mik., *Nowickia marklini* Zett., *Echinomyia praeceps* Mg. (and its subspecies), *Echinomyia lefeburei* R.-D., *Peletieria nigricornis* Mg., *P. ferina* Zett., *Cuphocera varia* F. and *C. ruficornis* Macq. are also closely related to *F. fera*. I have studied the ♂ and ♀ terminalia of *Peletieria nigicornis* Mg. and can confirm this conclusion ; this species appears to be a primitive member of this group. Unlike Baranoff, however, I consider the best way to classify these species is to place them in the single genus *Echinomyia* and to divide them into groups according to their relationships to each other ; the seven species dealt with in this paper belong to the *fera* group ; it is of minor importance whether it be given the status of a subgenus or a group. I am certain that there are many more species which belong to the genus *Echinomyia* in its wide sense, and these will only be discovered by comparative studies of the ♂ and ♀ terminalia of species of many genera. It is not possible, therefore, at present to express any opinion on the final limits of the genus. But, together with allied genera yet to be discovered, I would place all these *Echinomyias* in a tribe, the Echinomyini.

This brings me to the question of what characters provide the most reliable clues to the grouping of species into genera in the higher Diptera. In considering this question it is necessary to point out that a genus is a purely arbitrary concept, and is based on the value attached by its author to certain external characters. My studies of the ♂ and ♀ terminalia of such genera as *Musca*, *Stomoxys*, *Calliphora*, *Gasterophilus*, *Hypoderma*, *Auchmeromyia*, *Cordylobia* and many others have demonstrated the fact that genera based on such external characters as hairs and bristles, the structure of the palp and arista, and even the venation, cut across the true relationships of species, thus giving a purely artificial classification. The structure of the phallosome, the paramere, and the ♀ terminalia, on the other hand, give us reliable clues to the grouping of species into genera, thus providing a natural classification. Of one point I am certain—that every now and then the worker will come across a species (especially in such a vast assemblage as the Tachininae) in which even the terminalia appear at first sight to offer practically no guide to the true systematic position of the species. It would be wise in such cases to defer attempting to classify such a species until the terminalia of many more possibly related forms have been studied. I have recently come across a remarkable example of this in my studies of the terminalia of the species of the genera *Auchmeromyia* and *Cordylobia*. Species which have been placed in isolated genera without any indication of their true relationships have turned out, as the result of the study of the terminalia, to be members of the genus *Cordylobia*. The discovery of a connecting link between the genera *Auchmeromyia* and *Cordylobia* has amply confirmed these conclusions.

I shall be grateful to any of my readers who can spare me duplicates (both sexes) of any of the following :—

Mikia magnifica	*Chaetopeletieria* species	*Chrysosoma* species
Nowickia marklini	*Sphysocera* ,,	*Ernestia* (sens. lat.) species
Micropthalma disjuncta	*Cuphocera* ,,	
Schineria tergestina	*Micropalpus* ,,	
Peletieria species	*Pokoryna* ,,	
Tessarochaeta ,,		

REFERENCE

Baranoff, N. (1929). Studien an pathogenen und parasitischen Inseckten. I: Die jugoslavischen Arten der Tachinidengruppe *Echinomyia*. *Inst. Hyg. u. Sch. Volksgesund., Zagreb, Arbeit. Parasitol. Abt.*, no. 1.

RESEARCHES ON BLACKWATER FEVER IN GREECE*

I.—INTRODUCTION AND HISTORY

BY

HENRY FOY

AND

ATHENA KONDI

(From the League of Nations Blackwater Fever Research Laboratory, Salonika, Greece)

(Received for publication 15 *July*, 1935)

INTRODUCTION

These researches are being undertaken within the aegis of the Health Organization of the League of Nations. They are being conducted as a more-or-less co-operative study in association with the School of Hygiene in Athens and its malaria research division under the control of the International Health Division of the Rockefeller Foundation. The present paper on the history of blackwater fever will be followed by others (now in the course of preparation) on epidemiology, laboratory and hospital studies, experimental work on blockade of the reticulo-endothelial system with thorotrast and electro-colloidal copper in general paralytics with blackwater fever, and on the relation of malaria and quinine in blockaded and non-blockaded cases.

With regard to the present paper, a certain amount of confusion seems to exist as to the precise interpretation of the Hippocratic cases, a few authors taking the view that they do not represent blackwater fever. A careful examination of the Hippocratic corpus in Greek, together with the translated texts of Kühlewein (1894), Mercy (1884), Littré (1839), Gardeil and Coray (1885), Jones (1923), Adams (1849), Desmars (1767), Van den Linden (1665) and Kühn (1825), leads one to the opinion that, if the cases described by Hippocrates are not blackwater fever, then it is very hard to say what they can be. Greek opinion is almost solid in regarding them as blackwater fever ; Manson-Bahr (1931) refers to them as blackwater fever ; and Thomson (1924) suspects that they are likely to be, but prefers to leave the question open.

No doubt a certain amount of confusion has arisen over the transcription and translations from the original Greek into Latin, German, French, Italian and English, and in some cases we have found that words or even parts of whole sentences have been cut from the original texts. In this respect we have found Littré to be an offender, and we have preferred to use the Kühn, Kühlewein,

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* These researches were made possible and have been supported by a research fund deposited with the Health Committee of the League of Nations by Mrs. David Simmons.

and Gardeil and Coray texts. In all cases, however, we have checked the texts by a reference to all the editions available, as quoted above. We have confined our attention to the ' Epidemics ' of Hippocrates, and have made no attempt to search or check the ' Humours ' or other works for cases. We believe that this is the first time that the Hippocratic texts have been critically analyzed with regard to these blackwater fever cases.

At the present time there appear to be four more-or-less ill-defined types of haemoglobinuria occurring in Greece, some of which can hardly be regarded as falling into the category of blackwater fever, as it is at present understood. One type is the so-called malaria haemoglobinuria (with or without quinine) ; another is produced as a result of eating beans, *Fava vulgaris* (cf. Macas, 1933 ; McCrae and Ullery, 1933 ; Lotti and Puxeddo, 1927 ; Stathopoulos, 1933) ; a third group consists of quinine-sensitive individuals ; and lastly there are a certain number of cases of Weil's disease, that pass most often as non-spirochaetal haemoglobinuria and are frequently diagnosed as blackwater fever in the usual meaning of that term. Clinically, the first three are indistinguishable from one another, and the fourth can only be satisfactorily clinched by laboratory methods, rarely resorted to in Greece.

Whether these various types of haemoglobinuria were existent when Hippocrates wrote in 450 B.C. is impossible to say, though it is highly improbable that the Greeks were familiar with quinine. It seems reasonable, however, to suppose that some of the cases which Hippocrates described have as much justification to be regarded as blackwater fever as have some of the cases appearing and being reported as such to-day. If certain of the cases of haemoglobinuria noted to-day do not fall into the category of blackwater fever, it is possible that the same state of affairs existed when Hippocrates wrote. Those authors who deny the Hippocratic cases, and yet at the same time accept all the present-day returns for blackwater fever from Greece without question (cf. Stephens, 1927), seem to take up a not altogether reasonable position. And there can be no question that the fidelity and conscientiousness of the Hippocratic case-taking is as great as, and in very many instances superior to, the present-day reports, as we have had every opportunity of judging.

That the Greeks were unfamiliar with quinine is hardly an argument against regarding the Hippocratic cases as blackwater, as some authors have suggested (cf. Jones, 1909).

Furthermore, certain errors have crept into the literature regarding the priority of those authors first mentioning blackwater fever in what may be called modern times, and these errors have been repeated from book to book and from paper to paper. It is hoped that references and translations from the original papers, which were themselves consulted, will help to clarify this confusion.

Our thanks are due to Dr. A. Kouzis, Professor of the History of Medicine in the University of Athens, and to the German, American and British Schools

of Archaeology, for their help in the very onerous task of references to ancient Greek and Byzantine literature.

HISTORY

Blackwater fever has been known to exist in Greece from the earliest times. The majority of the Greek literature attributes the first reference to Hippocrates (' Epidemics '). Manson-Bahr (1931) and Thomson (1924), as already mentioned, incline to the same view, but in the absence of more precise information the latter prefers to make no definite statement. Jones (1909), on the other hand, says that ' one of our best authorities on the disease [name not mentioned] assures me that the cases described in the first book of the *Epidemics* cannot be blackwater fever. . . . A theory is at present much in vogue that traces its origin, at least in many cases, to the use of quinine, with which the Greeks were certainly unfamiliar.' Such a passage leaves one definitely with the impression that the reason for not regarding the Hippocratic cases as blackwater fever was that the Greek of the time of Hippocrates had no quinine. The present uncertain state of our knowledge with regard to the precise relationship of quinine in the genesis of blackwater fever makes such a statement of somewhat doubtful value. As already mentioned, the consensus of Greek medical opinion (Cardamatis, 1900 ; Antoniades, 1858 ; Kouzis, 1908 ; Alexandrides, 1932 ; etc.) is in favour of regarding the cases mentioned by Hippocrates as blackwater fever rather than anything else ; and, judging from the number of cases reported from Greece (cf. Stephens, 1927), medical men in Greece are probably more familiar, and have had a longer acquaintance, with the disease than most others, and their opinion should therefore be of value.

Stéphanos (1884), referring to blackwater fever in Greece, says that the cases described by Hippocrates from Thassos were probably blackwater fever. He makes the further statement that ' haemoglobinuric malaria ' was relatively rare or unknown in many places in Greece during the first years of independence, while at the time when he was writing it was rather frequent. Stéphanos quotes Antoniades as giving the first reference to blackwater fever in modern times. Turning to distribution, Stéphanos says that it was common in his time (as now) in the Sperchios valley in Thessaly ; that cases appeared at all times, but that large numbers occurred in the malaria epidemic of 1858-59 and 1864-66. Thomson (1924) asserts that ' blackwater fever has been known since the time of Hippocrates,' but adds that no definitely recognizable description of haemoglobinuria was published till the middle of the 19th century. Commenting on Greece, Thomson gives Veretas (1859) as the first reference, followed by Konsolas. There is no question from an examination of the Greek literature in the original that Antoniades (1858) has precedence over Veretas, and that the latter's paper followed as a result of Antoniades' previous one. Regarding Konsolas, a thorough search of the literature fails to reveal any work by this

author either on blackwater fever or any other subject. All references (Thomson, 1924 ; Cardamatis, 1900) to work by Konsolas refer to a paper by Veretas (1859), on page 30 of which Veretas makes a statement that ‘ Konsolas told me,’ etc.

Returning to Hippocrates, below are given a number of representative cases freely translated into English either from the Greek, the German or the French, and checked against the already existing English texts. It will be observed that the various cases exhibit such characteristic symptoms of blackwater fever as black or red urine, icterus, enlarged spleen, vomiting, anuria, uraemia and fever.

Case 1. Epidemics, Book I (1st Series).

‘ Philiscus, who lived by the Wall, took to his bed with acute fever. Towards night he sweated and was uneasy. On the 2nd day all symptoms exacerbated. Late in the evening, as a result of a suppository, he passed a normal stool ; had a quiet night. On the 3rd day from early morning till noon he appeared to be free from fever, but towards the evening acute fever developed, with sweating, thirst and a parched tongue ; passed black urine [*μέλανα οὖρα*] ; night uncomfortable ; no sleep ; completely delirious. On the 4th day all symptoms exacerbated ; urine black ; more comfortable night ; urine of a better colour. On the 5th day, about noon, had a slight trickling of pure blood from the nose (epistaxis) ; urine varied in character, having floating in it scattered bodies which did not sediment, and which resembled semen. A suppository was given, and a scanty flatulent stool was passed ; night uncomfortable ; little sleep ; irrational talking ; extremities quite cold, impossible to warm them ; urine black ; towards morning he slept a little ; loss of speech ; cold sweats ; extremities livid. About the middle of the 6th day he died. The respiration throughout was infrequent and deep, and like that of a person recollecting himself.* The spleen was swollen in the form of a round tumour ; cold sweats throughout ; the paroxysms on alternate days.’

Note :—*Fever* ; *black urine* ; *epistaxis* ; *Cheyne-Stokes respiration.* (Galen comments on this case and says that a fatal issue could be predicted when the fever returned on the 3rd day, with the complications of thirst and black urine.)

Case 2. Epidemics, Book I (1st Series).

‘ Silenus lived on the broadway, near the house of Evalcides. From fatigue, drinking, and unseasonable exercises he was seized with fever, which commenced with pains in the loins, stiff neck and a heavy head. On the 1st day the alvine discharges were bilious, unmixed, frothy, highly coloured and copious ; urine black with a black sediment ; he was thirsty, with a parched tongue ; no sleep at night. On the 2nd day acute fever, stools more copious, thinner and frothy ; urine black ; an uncomfortable night with slight delirium. On the 3rd day all the symptoms exacerbated ; soft oblong distension stretched on either side of the hypochondrium to the navel ; stools thin and darkish ; urine turbid and darkish ; no sleep at night ; much talking, laughing, singing ; no control over his emotions. 4th day same state. 5th day stools bilious, unmixed, smooth, greasy ; urine thin and transparent ; lucid intervals. On the 6th day slight perspiration about the head ; extremities cold and livid ; much tossing about ; no passage from the bowels ; urine suppressed (*οὖρα ἐπέστη*). On the 7th day, loss of speech ; extremities could not be warmed ; no discharge of urine. On the 8th day a cold sweat, with which appeared a red rash like small lentils which did not subside on slight pressure ; copious discharges from

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*The patient seemed to forget the necessity of breathing, and then to remember it and to breathe consciously. It seems clear that Hippocrates is describing uremic respiration (i.e., Cheyne-Stokes respiration).

the bowels of a thin and undigested character, painful to pass ; urine acrid and passed with pain ; extremities slightly warmer ; sleep fitful ; coma ; speechless ; urine thin and transparent. On the 9th day same state ; 10th day no drink taken ; comatose ; light sleep ; alvine discharges the same ; urine abundant and thickish, a white farinaceous sediment settled on standing ; extremities again cold. On the 11th day he died. From the beginning of the illness to the end the respiration was infrequent and deep ; there was a constant throbbing in the hypochondrium. His age was about 20 years.'

Note :—*Fever* ; *black urine* ; *enlarged spleen* ; *anuria* ; *respiration.*

CASE 2. EPIDEMICS, BOOK III.

' Hermocrates, who lay sick by the New Wall, was seized with fever. He began by having pains in the head and loins ; a slight distension of the hypochondrium ; tongue at first was parched ; deafness at the commencement ; no sleep ; not very thirsty ; urine thick and red [*οὖρα παχέα ἐρυθρά*], did not subside on standing [= had no sediment ?]. Stools not scanty, but burnt [very dry ?]. 5th day urine thin, had substances floating in it which did not subside ; at night was delirious. On the 6th day jaundiced [*ἰκτεριώδης*] ; all symptoms exacerbated ; was not rational. On the 7th day in a very uncomfortable state ; urine thin, as formerly. On the following days the same. About the 11th day there seemed to be general relief. Coma set in ; urine thicker and reddish, thin below, had no sediment. By degrees he became rational. On the 14th day fever gone ; had no sweat ; slept ; reason returned ; urine of the same character. About the 17th day he had a relapse, and became hot. On the following days acute fever ; urine thin ; was delirious. Again on the 20th day had a crisis ; free of fever ; no sweats. No appetite throughout the whole time ; was perfectly collected but could not speak ; tongue dry, without thirst ; fitful sleep ; coma. About the 24th day he became heated ; bowels loose, with thin watery discharge. On the following days acute fever ; tongue parched. Died on the 27th day. In this patient, the deafness continued throughout ; the urine was either thick and red without sediment, or thin, devoid of colour and having substances floating in it. He could not eat.'

Note :—*Fever* ; *enlarged spleen* ; *black urine* ; *icterus* ; *coma.*

CASE 3. EPIDEMICS, BOOK III (2ND SERIES).

' In Thassos, Pythion, who was ill above the Temple of Hercules, was seized with acute fever, and a strong rigor, as a result of hard labour and neglected diet. Tongue dry ; thirsty and bilious ; had no sleep ; urine darkish with suspensions floating in it which did not subside. On the 2nd day, about noon, extremities cold, especially the hands and feet and head ; loss of speech and articulation ; breathing short for a long time ; recovered his heat ; thirst ; quiet night ; slight head sweats. 3rd day, in the evening about sunset, slight chills ; nausea ; distress ; painful night ; no sleep ; small compact stools. 4th day, early morning, peaceful, but at noon the symptoms became exacerbated ; coldness ; loss of speech and articulation ; became worse ; recovered his heat ; after a time passed black urine, having substances floating in it ; night peaceful ; slept. On the 5th day seemed relieved, but heavy and painful belly ; thirsty ; restless night. 6th day, early morning, peaceful ; pains greater ; towards evening, exacerbation ; later a light enema was given and bowels opened well. Slept at night. On the 7th day in a state of nausea, and disturbed ; passed urine like oil ; at night much agitated ; was incoherent ; no sleep at all. On the 8th day slept a little in the morning, but immediately got cold ; loss of speech ; respiration weak and shallow ; in the evening was warmer ; delirium ; towards morning was slightly better ; stools unmixed and bilious. 9th day, coma, with nausea when raised ; not very thirsty. At sunset became restless and incoherent ; passed a bad night. On the tenth morning he became speechless ; very cold ; acute fever ; much perspiration. He died ; his sufferings were on even days.'

Note :—*Fever* ; *black urine* ; *vomiting* ; *respiration.*

CASE 9. EPIDEMICS, BOOK III (2ND SERIES).

' In Abdera, Heropythus, while up, had pains in the head, and shortly afterwards went to bed. He lived near the High Street. Was seized with acute fever of the ardent

type ; vomiting at the beginning much bilious matter ; thirst ; great restlessness ; urine thin and black, with substances floating near the surface, but sometimes not. Painful night ; varied and irregular paroxysms of fever. 14th day deafness ; fever increased ; urine the same. On the 20th day and following much delirium. On the 40th day epistaxis ; became more collected ; some deafness ; fever less. Frequent but slight epistaxis on the following days. About the 60th day haemocharges ceased, but violent pains in the right hip and an increase of fever. Shortly afterwards pains in the whole lower part of the body. It then happened that either the fever and deafness increased, or if these abated the pains in the lower part of the body grew worse. About the 80th day all symptoms relieved, but not disappeared. The urine was a good colour and had a copious sediment ; delirium less. About the 100th day copious bilious evacuations which continued for a considerable time, assuming a dysenteric form, and somewhat painful. On the whole the fever disappeared, the deafness ceased. On the 120th day complete crisis.'

Note :—*Vomiting* ; *fever* ; *black urine* ; *epistaxis*.

Case 13. Epidemics, Book III (2nd Series).

'Apollonius in Abdera bore up [under the fever ?] for some time and would not go to bed. Viscera [spleen and liver] enlarged ; continuous pain about the liver for a long time ; became icteric, and was flatulent, with a whitish complexion. Having eaten beef and drunk unreasonably, he became heated and betook himself to bed, taking large amounts of milk both sheep's and goat's, boiled and raw ; this diet did him great mischief, exacerbating the fever. Little or none of the food taken was evacuated ; urine thin and scanty ; no sleep ; troublesome flatulence ; much thirst ; comatose ; painful swelling in the right hypochondrium ; extremities coldish ; incoherent ; unconsciousness of all he said. The 14th day from his going to bed, he had a rigor ; grew heated ; maniacal delirium ; loud cries ; much talking, followed by calm ; the coma came at this time. Bowels disordered ; copious bilious stools of undigested faeces ; urine black, scanty and thin. Very restless. Varied alvine discharges, either black, scanty and verdigris green, or fatty, undigested and acrid. 24th day calm ; other symptoms much the same ; he had lucid intervals of memory since he took himself to bed, but he was soon delirious again, and all the symptoms became worse. 30th day acute fever ; stools copious and thin ; delirium ; extremities cold ; loss of speech. Died on the 34th day. In this case so far as I saw the bowels were disordered, the urine thick and black ; disposition to coma ; insomnia ; extremities cold ; delirium throughout.'

Note :—*Fever* ; *icterus* ; *enlarged spleen* ; *coma* ; *scanty black urine*.

From 450 B. C. till late Byzantine times, no reference to black urine or to any other system that may be regarded as relating to blackwater fever is traceable. In the 7th century A. D., however, Theophilos Protospatharios mentions black urine, stating that ' with the evacuation of this black urine the fever leaves the body and leads to a dissolution of the melancholia.' In the 12th century A. D., Actuarios, the last of the Byzantine medical writers, refers to black urine accompanied by fever and icterus. Whether these two writers can be regarded as referring to blackwater fever is difficult to judge ; but in the cases mentioned by Actuarios the black urine is described in conjunction with icterus and a fever that may have been malaria.

From Actuarios's time till the middle of the 19th century, no statements are traceable in Greek that may be regarded as referring to blackwater fever, though certain Italian writers, with whom this paper is not concerned, at the end of the 18th century are reported to have noted ' haematuria ' following the administration of quinine. (See below.)

In 1842 Mavroyanis, giving an account of the distribution and incidence of various diseases in Greece says that ' one form of malignant intermittent fever is that which affects the bowels, namely, the bloody or black-bile fever.'* Whether this description is meant to apply to haemoglobinuria seems doubtful.

The first undoubted reference to blackwater fever in Greece by a Greek author was in 1858, when Antoniades published his paper ' Concerning Haemorrhages and Haematuria.'† In this paper, he mentions that ' a number of Greek doctors have observed that haematuria [= haemoglobinuria] is a common symptom of the intermittent fever and follows the administration of quinine, and they conclude that this haematuria is due to quinine poisoning or to an idiosyncrasy that some individuals possess for this drug.' Antoniades in his paper places no credence in the quinine theory of ' haematuria,' although he does consider that some individuals may be sensitive to this drug ; he prefers, however, to leave the question an open one until more substantial proof is forthcoming. He concludes his paper by citing cases of haematuria which he treated with quinine, and says : ' I am emphatic that the treatment of haematuria is the same as for all the intermittent fevers, namely, quinine, and I regard the withholding of quinine, in cases of haematuria, as criminal negligence.'‡ With this paper may be said to commence in Greece the controversy as to the relation between quinine and blackwater fever, which has continued almost without interruption till the present day.

In the same year (1858), probably as a result of having read Antoniades' paper, Veretas read a paper (November 6th, 1858) before a branch of the Greek Medical Association in Paris, which was published in 1859. His paper was entitled ' Haematuria in the Intermittent Fevers and its Relation to Quinine.' Here Veretas gives his views as to the genesis of haematuria, quoting in his support his own father and Konsolas.§ His views are in sharp contrast to those of Antoniades and the latter's supporters. In the opinion of Veretas, some individuals are sensitive to quinine (he mentions his own father as a case in point), and quinine, when administered in cases of intermittent fever, provokes haematuria. He quotes Rubini as having first observed in 1779 ' urinary symptoms ' following the administration of quinine, and Faginoli from Verona as having noted ' urethral irritation and drops of blood in the urine ' following quinine. Veretas gives the histories of two cases, in addition to that of his father, in which the administration of quinine was followed by ' haematuria ' ; and he sums up his cases and states his position by saying that ' none of these

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*' *Εἰς τούς κακοήθεις κυρετούς τουςκροσβάλλοντάς τά σπλάχνα τά κοιλιακά ὑ-κάγεται καί ο' αι'μάτηρός ή μελανόχωλερικός.*'

† Haematuria is the term used at the period to describe what is now known as haemoglobinuria ; no distinction was drawn between R.B.C. in the urine and simple Hb.

‡ It is interesting to note that the great majority of cases of blackwater fever occurring to-day in Greece are treated by massive doses (1 gm.) of oral, intramuscular or intravenous quinine.

§ See page 386.

patients ever had haematuria from fever alone, but only after quinine ; . . . and from this material it is clear that in our climate quinine acts on some sensitive individuals, irritating the urinary system, and bringing haematuria.'

In 1861 Antoniades presented a second paper, ' Malignant Fever with Circulatory Symptoms.' In this somewhat carefully reasoned paper, he describes several types of intermittent fever (M.T.) which affect the vascular system, producing haemorrhages which include epistaxis, haemoptesis, haematemesis, intestinal haemorrhages, haematuria, etc. In describing the ' haematuric type ' (haemoglobinuria), Antoniades refers to Hippocrates as having observed cases. He then describes the characteristic symptoms of blackwater fever, and says that ' many authors have imagined that this haematuria is the result of the administration of quinine sulphate, because the patient had taken quinine previous to the passage of black urine.' He then states that he treated a relapsing case of blackwater fever with one dose of quinine and found it to be efficacious in controlling the second attack. Referring to his previous paper he says : ' In that paper I give examples and reasons which make it clear that the fever [malaria] and not quinine is the cause of the haematuria, because in other diseases quinine when administered does not produce haematuria.' He further states that the haematuria described by foreign authors (Faginoli and Rubini) is different from that of Greece, ' because in the Greek cases there is no inflammation or pain in the bladder, no urethral stricture or haemorrhage . . . and for this reason I insist that the haematuria of the intermittent fevers in our climate is not the result of quinine, but of malaria itself.' Continuing, he says : ' If there prove to be cases in which quinine does produce haematuria, then we must imagine that this is due to an increase in the blood supply to the kidneys caused by the quinine, thus facilitating haematuria ; but it must not be imagined that quinine is the actual or direct cause of haematuria.' He concludes his paper in the same vein as his former one, by emphasizing that the treatment for this haematuria is quinine.

With the publication of this paper, both Antoniades and Veretas appear to drop out of the arguments ; but Veretas's views are ably defended in 1861 by Papavassiliou, writing on ' Five Cases of Haematuria Provoked by Quinine.' In these five cases the author describes typical cases of haemoglobinuria occurring from 1–8 hours after doses of quinine, varying from 7–24 grains, which had been given for intermittent fever. In one of the cases the patient had previously been in the habit of taking quinine for malaria, and no untoward symptoms had developed ; but at the time described, quinine, when given in a dose of 24 grains, resulted in the development of haemoglobinuria. Papavassiliou makes no comment on his cases, and there is nothing especially atypical about them.

In 1872, Rizopoulos published a somewhat vague paper, ' Concerning Bilious Haematuric Fever,' in which he sets forth his views with regard to the origin of blackwater fever. He expresses his belief that ' bilious haematuria '

is not due to quinine (although he confesses that he formerly held this view), but to ' the effect of a moist tropical climate on the gastrohepatic system, and to an unusual energy of the biliary system.' Then follows a long account of various cases of haemoglobinuria, some of which developed after quinine had been given for malaria, some where it did not, and some again where he treated ' haematuria ' by means of quinine. He concludes his paper by stating that in his opinion haematuria is due to kidney damage and not to quinine, and he expresses his belief that the haemorrhages occur always in the kidney. He does not subscribe to the idea of quinine-sensitive individuals, as does Veretas.

The next paper to appear came from Karamitsas in 1878, entitled ' Haematuria or Haemoglobinuria from Quinine.' It is in this paper that the disease is first referred to as haemoglobinuria, as opposed to the haematuria of the previous authors, and Karamitsas states that the urine contains only haemoglobin. In this paper the author deals with the whole problem of haemoglobinuria as he visualized it at that time. He gives a brief history of the views of the previous authors, including Veretas, Antoniades and Rizopoulos. He refers to a previous paper of his own, published in 1874, in which he observes that ' I have had several cases of simple haematuric intermittent fever treated with quinine, but I have also seen patients with intermittent fever who when given quinine passed bloody urine.' In Niemeyer's ' Medical Pathology,' vol. 2, p. 875, Karamitsas wrote : ' In some patients who have intermittent fever, quinine provokes haematuria, which must be distinguished from the haematuria that follows it [the fever ?] without quinine, and the urine must be examined to discover whether blood or haemoglobin is present.'

Karamitsas says that he firmly believes that there are cases of haemoglobinuria that follow intermittent fever, and that these cases can be safely treated with quinine. He further states that he has seen ' cases of haematuria that follow the administration of quinine,' and that in such cases it is better to withhold quinine. Later, however, he says : ' I have never seen haematuria in non-malarious places, even though quinine be taken.' He examines in some detail the question whether frequent doses of quinine taken over long periods, and in association with malaria, can produce quinine sensitiveness. His opinion on this point is in the negative, but his further remarks are worth quoting : ' I do not think so,' he says, ' since there are many people who take frequent large doses of quinine over long periods and are also affected with chronic malaria, but by no means all develop haematuria. In certain individuals, who take frequent small doses of quinine, symptoms develop, presumably due to quinine, that may resemble malaria, such as urticaria, formications, haemorrhages, tremblings, spasms, etc. . . . I am of the opinion that quinine can produce icteric haematuric fever. I know of a case of quantana fever who, whenever he took quinine, developed haematuria and icterus.' From an analysis of Karamitsas's papers it is very hard to gather with any certainty what position he actually does take up with regard to the relationship of quinine to the genesis

of blackwater fever. Whether he regards both haematuria and haemoglobinuria, or only one or the other, as caused by quinine, and whether malaria is a necessary concomitant of the quinine in either or both diseases, is impossible to say. He does, however, state that in his opinion there are two types of haemoglobinuria, one caused by quinine and the other by malaria.

From Karamitsas to the present day there are innumerable references to blackwater fever and the relation of quinine and malaria to the disease, but no purpose would be served by an analysis of this later work.

SUMMARY

This paper is the first of a series of studies on blackwater fever in Greece, and deals with the history from the time of Hippocrates up to 1878.

The purpose of the present paper is to call attention to what appear to be undoubted cases of blackwater fever described in the ‘ Epidemics ’ of Hippocrates. The Greek texts of the Hippocratic corpus have been examined, together with the German, French, Latin, Italian and English translations.

Certain priority errors are examined and corrected, and the references and translations from the original Greek are given, in the hope that the confusion may be to some extent cleared.

References to black urine, icterus, fever and enlarged spleens are found among the later Byzantine writers.

Mavroyanis in 1842 refers to the ‘ bloody or black-bile fever.’

Antoniades in 1858 gives the first unquestioned account of blackwater fever since Hippocrates, and this is followed by an account by Veretas in 1859.

Attention is directed to four ill-defined types of haemoglobinuria occurring in Greece, the presence of which complicates studies here, and tends to upset epidemiological data unless taken into account.

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RESEARCHES ON BLACKWATER FEVER IN GREECE

II.—A NOTE ON THE ACTION OF EHRLICH'S DIAZO-REAGENT ON THE UNHAEMOLYZED SERUM OF THE RABBIT

BY

HENRY FOY

AND

ATHENA KONDI

(From the League of Nations Blackwater Fever Research Laboratory, Salonika, Greece)

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During the course of experimental work on malaria in rabbits and the blockade of their reticulo-endothelial systems with electro-colloidal copper (Heyden),* it was thought desirable to ascertain to what extent the blockade had become effective, since it would appear to be impossible to draw conclusions from such work unless some measure of the degree to which blockage had progressed was available. To do this it was decided to determine the respiratory output of the minced liver and spleen cells, using the method of Fenn (1927), as recently modified by Ramsey and Warren (1930), and checking this on a second sample of cells by the method of Jungeblut and Berlot (1926). Since both these methods involve the slaughter of the animals, it was decided, in those cases where it was felt that the blockade had not progressed very far, to estimate the effect of the partial blockage on the serum bilirubin on animals injected with a haemolysin. Obviously the first step in such work was to ascertain the bilirubin level in normal unblocked animals, so as to establish a standard for comparison ; and the following is an account of the work carried out with this end in view. A full report of the work on blockade of the R.E.S. and its effects on blood quinine levels, and thence on the disappearance of parasites from the blood, will be given later ; here it is only desired to call attention to the results obtained by applying the Van den Bergh reaction to the serum of rabbits. Blood was obtained from the rabbits by means of heart puncture, without the use of any anticoagulant (since it was found that this tends to produce some slight haemolysis). The blood thus obtained was gently centrifugalized at about 1,500 revolutions per minute. The clear serum was found to be absolutely free from any haemoglobin by spectroscopic analysis. This haemoglobin-free serum was divided into two parts : on one part, the original Van den Bergh technique (cf. Harrison, 1930) was applied, of precipitating the

*This substance was courteously supplied by Messrs. von Heyden, Dresden, and was made according to the formula of Jancsó.

proteins first, centrifuging, and then adding the diazo-reagent to an aliquot portion of the supernatant liquid ; to the other portion of the serum the technique of Thannhauser and Andersen (1921), as modified by Hunter (1930) and White (1932), was applied. In neither case was it possible to detect any reaction whatever, either direct or indirect. The reason for applying both these techniques was that, during the course of investigations on the bilirubin content of the serum of cases of blackwater fever, it was found that the results obtained by the use of both these methods on the same blood was apt to give results that differed by sometimes as much as 50 per cent., especially when the bilirubin content was low. The reason for this would appear to be, in some cases at least, the development of a brownish tinge in the unknown, which made the comparison with the standard a somewhat arbitrary affair. To some extent this difficulty is overcome by using the suggestion of Nichols and Jackson (1930), and by preparing the cobalt standard according to the formula of White (1932) ; but when the concentration of bilirubin is very low this does not suffice. That there are other sources of error in addition to this discolouration was shown spectro-photometrically by means of a Pulfritch Stufenphotometer, when differences up to 50 per cent. were encountered when the two methods were applied to the same blood. Sometimes one technique gives higher results and sometimes another, irrespective of the amount of haemolysis.

Feeling that blood from the heart might, in some not understood way, affect the bilirubin content as a result of direct passage from the lungs (since it was uncertain from which ventricle the blood had been drawn), it was decided to use vein blood, as is usual in the Van den Bergh reaction. A technique was developed which permitted the withdrawal of 5–10 c.cm. of blood from one of the ear veins without haemolysis, as shown by spectroscopic examination. Both the original and the modified Van den Berghs were applied to the serum thus obtained, with, however, the same negative results as for heart blood. The rabbits used were all non-pregnant females, fed on a normal diet of grain and greens, and kept under healthy conditions of captivity. No attempt was made to estimate the alkali reserve, and this may conceivably have some effect on the reaction (cf. M'Gowan, 1930). In all cases the test was carried out within ten minutes of drawing the blood.

Contrary to the work of Lepehne (1919), we find that the serum of normal rabbits contains no bilirubin that reacts with the diazo-reagent of Ehrlich, thus confirming Hida (1922), Yamanaka (1926) and Maeda (1932). How far this is due to smaller amounts present in the serum of rabbits, or to differences in the renal thresholds for this substance (cf. Rabinowitch, 1932), or to other factors connected with the reticulo-endothelial system, is impossible for us to say. Saiki (1930) has shown that injected bilirubin is excreted more easily into the urine of dogs than of rabbits, but no suggestion can be made as to the bearing of this observation on the present work.

SUMMARY

A series of experiments were performed on the unhaemolyzed serum of the rabbit, with the object of ascertaining the bilirubin level as a guide to R.E.S. blockade. Both the original Van den Bergh and the modified Thannhauser and Andersen technique were used. Contrary to the view of Lepehne, we find it impossible to detect any bilirubin in the serum of rabbits.

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